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Organochlorine, Trace Elements and Metal Contaminants in the Food Web of the Lightfooted Clapper Rail, Upper Newport Bay, California

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Santa Ana Regional Water Quality Control Board

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EXECUTIVE SUMMARY

Upper Newport Bay (UNB) is an ecological reserve of the California Department of Fish and Game and serves as refuge, foraging areas, and breeding grounds for a number of federal- or state-listed threatened and endangered species, including the Light-footed Clapper Rail or clapper rail (*Rallus longirostris levipes*). In 1996, the Santa Ana Regional Water Quality Control Board (SARWQCB) placed UNB and portions of San Diego Creek on the federal 303(d) list of impaired water bodies due to heavy and trace metal contamination (copper, cadmium, lead, selenium and zinc), pesticides (chlorpyrifos, dieldrin, chlordane, DDT) and PCBs. The many species of birds that nest or feed in Upper Newport Bay are an important receptor for these contaminants. One of the key steps in estimating the risk of contaminant exposure to birds is to determine the contaminant concentrations in various components of their diet and surrounding environment as well as direct measures of exposure (ie. bird eggs). This study is providing data to support the development of the Toxics TMDLs in the San Diego Creek watershed by determining the concentration of selenium (Se), heavy metals, and organochlorine compounds in the food web of the clapper rail. The objective of this study is to: 1) determine the concentration and degree of bioaccumulation of heavy and trace metals (including selenium), and organochlorine compounds in three components of the Upper Newport Bay ecosystem: non-viable clapper rail eggs, benthic macrofauna, and sediments and 2) evaluate contaminant impacts on clapper rails by examining nonviable eggs for evidence of egg shell-thinning or embryo developmental abnormalities that have been linked to these contaminants.

This study found that DDTs (including 2,4'-DDT, 4,4'-DDT and its metabolic products 2,4'-DDD, 2,4'-DDE, 4,4'-DDD, and 4,4'-DDE), technical chlordane compounds, selenium, and mercury are present and biomagnifying in the food web of the Light-footed Clapper Rail in Upper Newport Bay. Of these compounds, 4,4'-DDE is the contaminant of greatest concern. The rationale for this finding lies in 1) DDE concentrations exceed screening levels for sediments and bird eggs and 2) embryonic abnormalities and eggshell thinning occur in a clapper rail egg that contains elevated DDE concentrations. Selenium concentrations in clapper rail eggs were below levels considered to impair reproduction. Because of the limited number of egg samples analyzed, it is recommended that additional data be collected to test the hypothesis that DDT, Se, and Hg may be causing ecologically-significant effects to rail reproduction.

Although rates of biomagnification of DDTs in clapper rails relative to prey organisms (126-874 fold) were high, DDT concentrations in prey organisms (0.75 – 32 ng g^{-1} wet weight (wt)) were well below levels that have been shown to be acutely toxic to clapper rails feeding on them (LC_{50} of 3.2- 3.8 $x10^5$ ng g^{-1} wet wt). This suggests that DDT contamination is not causing avian mortality in Upper Newport Bay. However, chronic impacts, such as reproductive impairment, genetic mutations, etc. can occur at levels several orders of magnitude below acute toxicity thresholds. DDT concentrations in some Upper Newport Bay samples exceed a risk-based dietary screening level for clapper rails (17 ng g^{-1} wet weight) computed from the EPA NOAEL for pelicans. However, DDT concentrations measured in the clapper rail prey organisms are below lowest observed adverse effect levels (LOAELs) for significant reproductive impairment in studies reviewed by EPA, including the LOAEL for pelicans, which appear to be the most sensitive of avian species (150 ng g^{-1} wet weight).

Screening values for chronic reproductive impairment from total DDT are not currently available for the clapper rail, so comparisons were made using screening values from other omnivorous aquatic birds when available. The maximum DDE concentration found in the egg from Nest Site 5 (1050 ng g^{-1} wet wt) is below that which caused reduced clutch size, decreased productivity and increased incidence of shell cracking in the white faced ibis (3000 ng g^{-1} wet wt); but 4,4'-DDE was found in all 6 clapper eggs at concentrations exceeding levels of the lower range of the level of concern for biotic effects in omnivore waterfowl (250 ng g^{-1} wet wt). This suggests that DDT could be causing some degree of reproductive impairment of clapper rails in Upper Newport Bay. Observation of minor eggshell thinning and developmental abnormalities in the rail egg with the highest DDE concentration further supports cause for concern that DDTs may be causing some degree of reproductive impairment of clapper rails in Upper Newport Bay. Indications are that impairment due to DDTs is likely declining; DDT concentrations in clapper rail eggs measured in this study are less than previously measured in UNB during the 1980s. In addition, UNB is currently home to the greatest number of clapper rail breeding pairs in southern California, a number that has nearly doubled from 103 in 1982 to 174 in 2004. Notably, clapper rails feed at a lower trophic level than shorebirds. Consequently, the results of this study should be considered in conjunction with results of other studies that provide data for higher trophic level feeders when developing TMDLs for the San Diego Creek watershed.

The lack of PCBs and chlordane in sediments, prey organisms, and the low levels in rail eggs indicates that these contaminants are not likely to be of concern in the food web of the UNB clapper rail population. Average concentration of total PCBs found in UNB clapper rail eggs (94 ng g^{-1} wet wt) was an order of magnitude below levels thought to be of concern for decreased hatching success in chickens – the bird species most sensitive to PCBs found to date. Levels of total PCBs found in UNB clapper rail eggs (94 ng g^{-1} wet wt) were an order of magnitude below levels thought to be of concern for decreased hatching success for Aroclor 1242 in chickens – the most sensitive bird species to PCBs found to date (870 ng g^{-1} wet wt). Levels of chlordane components and metabolites in rail eggs (30 ng g^{-1} wet wt) did not exceed "no effect" thresholds. All other organochlorine compounds were at non-detectable concentrations in sediments, prey organisms and eggs[1].

The majority of sediment samples from the five nest sites had Se concentrations which exceeded levels considered to be of substantive risk to aquatic life (> 4 mg kg^{-1} dry wt); Se concentrations in prey organisms (3.6-9.8 mg kg^{-1} dry wt) in several cases exceeded projected dietary screening values indicating a substantive risk to fish and wildlife (> 7 mg kg^{-1} dry wt). Despite the fact that UNB rail egg Se concentrations were elevated relative to those in other southern Californian salt marshes, Se concentrations in UNB rail eggs were below threshold of marginal risk to aquatic birds (<6 mg kg^{-1} dry wt). In the egg that exhibited abnormal development from Nest Site 5, selenium toxicity did not appear to be the likely cause. The reason for this conclusion is due to the relatively low selenium concentration in the egg and lack of apparent deformities in the embryo that are characteristic of selenium, such as reduced or missing eye. While a combined effect of DDE, Se, and/or other contaminants may be responsible for the deformity, it is not possible to definitively determine the cause.

[1]Minimum detection limits for chlordane, toxaphene and dieldrin were below numeric TMDL targets established for sediments in the San Diego Creek watershed.

Of other trace elements, metals, and metalloids, Hg was the only contaminant whose concentrations were sufficiently elevated in UNB sediments, prey organisms and rail eggs to be of concern. Hg, as well as several other elements (Co, Cu, Pb, Ni, Ag and Zn), had sediment concentrations exceeding threshold effects levels (TEL) values. Only Hg, however, exceeded dietary screening levels in three of the primary prey organisms of the clapper rail (crabs, isopods, and snails; 0.15-0.2 mg Hg kg^{-1} dry wt). Mean Hg concentration in UNB clapper rail eggs (0.64 mg kg^{-1} dry wt) exceeded the no effect level, but only one egg (1.3 mg kg^{-1} dry wt) was within the lower range of the level of concern. Based on these results, it is highly unlikely that Hg is impairing clapper rail reproduction in UNB.

Due to the exclusive use of non-viable eggs, these results present a worst-case scenario appropriate for a screening level study. Because of this issue, along with the limited sample size and extent of spatial sampling in the estuary, we recommend further study to determine the extent to which DDTs, mercury and selenium may be currently impairing reproduction of the clapper rail in Upper Newport Bay. Such a study would complement ongoing studies examining organochlorine contamination in the food webs of UNB fish and fish-eating birds.

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INTRODUCTION

Newport Bay (Bay) is a shallow 752-acre estuary situated at the base of the San Diego Creek watershed. As the second largest estuarine embayment in southern California, it provides important natural habitat for terrestrial and aquatic species. The Bay provides significant spawning and nursery habitats for commercial and non-commercial fish species and is an important resting and feeding area for migrating birds on the Pacific Flyway. Upper Newport Bay (UNB) is a state ecological reserve and serves as refuge, foraging areas, and breeding grounds for a number of federal- or state-listed threatened and endangered species. Among these include the Brown Pelican (*Pelecanus occidentalis*), Peregrine Falcon (*Falco peregrinus anatu*m), Belding's savannah sparrow (*Passerculus sandwichensis beldingi*), Coastal California Gnatcatcher (*Polioptila californica californica*), the California Least Tern (*Sterna antillarum browni*), and the Light-footed Clapper Rail (*Rallus longirostris levipes*). The primary freshwater input to the Bay, San Diego Creek, provides a corridor for wildlife movement between the Bay and upland areas. These diverse habitats make the Bay an important ecosystem within the urban landscape of southern California (Figure 1).

Land use changes in the San Diego Creek watershed have led to increased freshwater and contaminant loads to the Bay; concurrently, there has been a decline in wildlife beneficial uses of UNB. Elevated concentrations of selenium are present in the water and organisms inhabiting San Diego Creek. Portions of Newport Bay are also contaminated by polychlorinated biphenyls (PCBs), and the chlorinated pesticides dichlorodiphenyltrichloroethane (DDT) and its breakdown product dichlorodiphenylethene (DDE) and dichlorodiphenyldichloroethane (DDD), chlordane, and dieldrin, as well as heavy metals such as cadmium, copper, lead, and zinc. Elevated concentrations of selenium and total DDTs (DDT+DDE+DDD) have been found in fish inhabiting Newport Bay and these contaminants have been identified as potential sources of water quality impairment in the Bay.

In the early 1990s, Newport Bay and San Diego Creek were placed on the CWA Section 303(d) list due to violations, or threatened violations, of Basin Plan narrative water quality objectives for toxic substances. In June 2002, USEPA promulgated technical TMDLs for heavy and trace metal contamination (copper, cadmium, lead, selenium and zinc), organophosphate pesticides (chlorpyrifos and diazinon), organochlorine pesticides (dieldrin, chlordane, DDT, and toxaphene) and PCBs for these water bodies. Under the current schedule, the RWQCB will be incorporating USEPA Technical TMDLs with an implementation plan into the Basin Plan, with some revisions based on updated knowledge of contaminant sources, fate, transport and cycling.

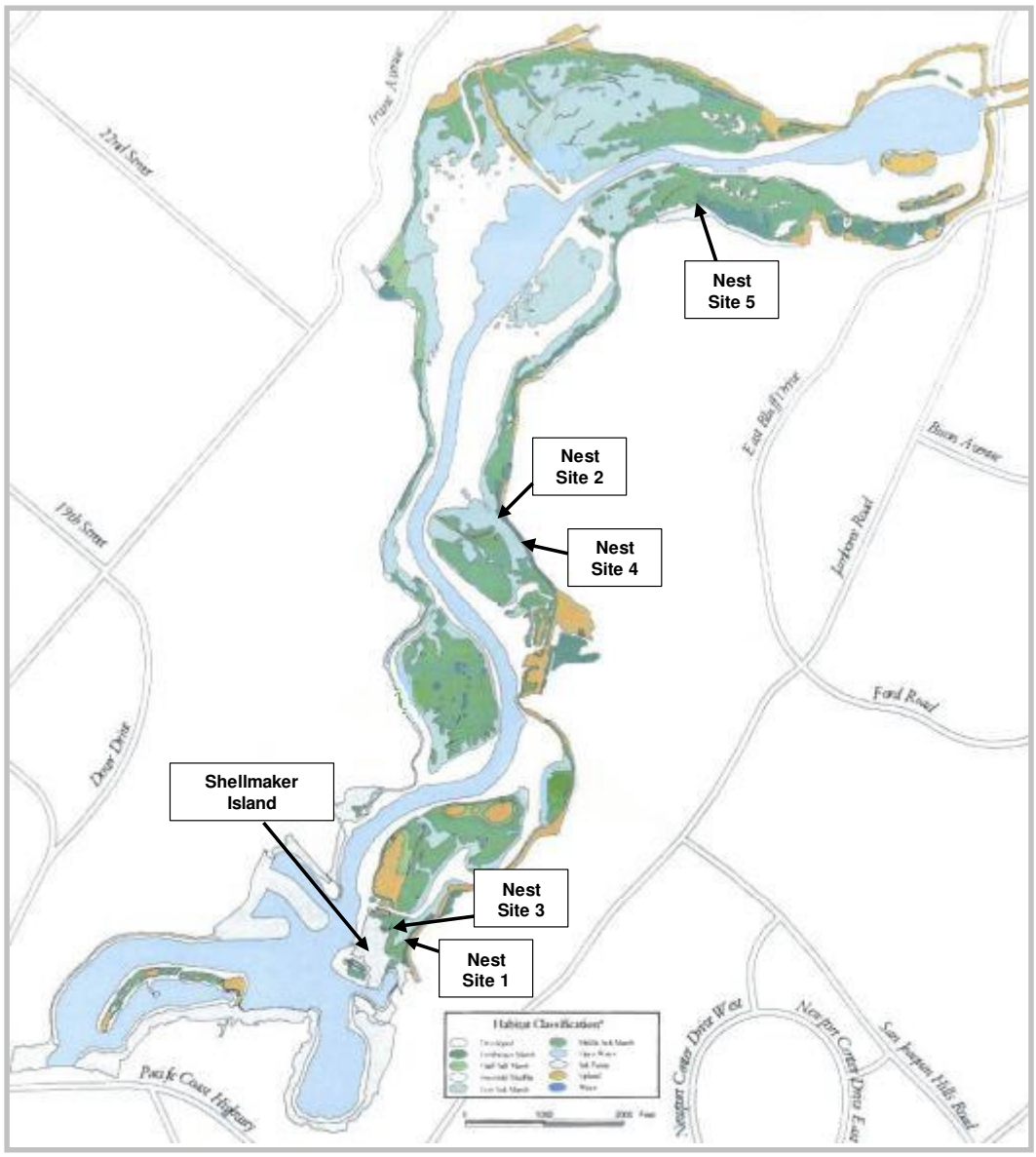

Figure 1. Map of Upper Newport Bay showing locations of clapper rail nest sites sampled (Base map credit USACOE UNB Restoration Feasibility Study 2001)

Development of a TMDL requires an understanding of the sources, fates, and effects of these contaminants in the bay. A recently completed study of contaminants in recreational and forage fish in Newport Bay found that DDTs, PCBs and some heavy and trace metals were bioaccumulating in fish above levels of concerns for human or wildlife fish consumption (Allen, Diehl et al. 2004). The many species of birds that nest or feed in Upper Newport Bay are also important receptors for contaminants in the Bay. One of the key steps in estimating the risk of contaminant exposure to birds is to determine the concentrations of the contaminants of interest in the various components of their diet, as dietary uptake is one of the major sources of exposure to many contaminants for wildlife, especially for compounds like selenium and organochlorines that bioaccumulate.

The goal of this study is to determine the concentration of selenium, heavy metals (cadmium, copper, lead, mercury, and zinc), and organochlorine compounds in the food web of a resident bird species. The Light-footed Clapper Rail (clapper rail) is a good candidate for the study of the bioaccumulation of toxic compounds in the Bay because, as a year-round resident of UNB, contaminants are not likely to be introduced by feeding and foraging in other estuaries. The clapper rail, found in the coastal marshes of southern California and northern Baja California, Mexico, has suffered a severe decline in population during this century, and is listed as an endangered species by both the federal (35 FR 16047, 16048) and state governments (CDFG 1972). The clapper rail nests in the lower intertidal zone of coastal salt marshes where dense stands of cordgrass (*Spartina foliosa*) are present (Massey, Zembal et al. 1984). They also build nests in pickleweed (*Salicornia virginica*) and have been known to reside and nest in freshwater marshes, although this is not common. They require shallow water and mudflats for foraging, with adjacent higher vegetation for cover during high water. The clapper rail nests from mid-March through July 1 and lays five to nine eggs between early April and early May. The nests are located in the salt marsh and are constructed of cordgrass or pickleweed. Clapper rails forage in the mudflats by shallow probing of the sediment or surface gleaning (Zembal and Fancher 1988). As omnivores, their diet consists primarily of small crabs, insects, insect larvae, worms, gastropods (snails and slugs), mussels, and small fish (Zembal and Fancher 1988). The clapper rail has been identified as one of the species in Upper Newport Bay that is at risk of immune system or reproductive impairment from dietary uptake of bioaccumulative compounds such as selenium and organochlorine compounds.

The objective of this study is to: 1) determine the concentration of heavy and trace metals (including selenium), and organochlorine compounds in three components of the Upper Newport Bay ecosystem: non-viable clapper rail eggs, benthic macrofauna (mussels, crabs, amphipod, isopods, snails, etc.), and the upper 2 cm of the sediment which contains detritus and nutrients that the benthic organisms feed on, and 2) examine eggs for evidence of biotic effects of contaminants such as egg shell-thinning or embryo developmental abnormalities. Benthic macrofauna and sediments represent key parts of the food web for many birds and fish. There are no recent data that describe the metals and organochlorine compound concentrations present in these types of samples. Obtaining information on the contaminant concentrations present in bird eggs and the corresponding food web components will help facilitate the estimation of the risk to Newport Bay wildlife from metals and organochlorine compounds.

METHODS

Site Description

Newport Bay is located in Central Orange County in the southwest corner of the Santa Ana River Basin, about 35 miles southeast of Los Angeles and 70 miles north of San Diego. Newport Bay is a combination of two distinct water bodies – Lower and Upper Newport Bay, divided by the Pacific Coast Highway (PCH) Bridge. The Lower Bay, where the majority of commerce and recreational boating exists, is highly developed. The Upper Bay contains both a diverse mix of development in its lower reach and an undeveloped ecological reserve to the north.

The total area of the reserve is 288 ha (752 acres) of which 100 ha (250 acres) is salt and freshwater marsh (Figure 1). The bay is long, narrow, and banked on both sides by steep cliffs. Several creeks and many small seeps along the bluffs provide year-round fresh-water influence. Stands of reeds (mostly *Scirpus sp*. and *Typha spp*.) line small ditches and ponds, and occur along much of the bay's edge. There is abundant low marsh with tall dense *Spartina foliosa* as the dominant plant. Inundation of cordgrass is never complete, even at high tide. The middle littoral zone is also well represented and diversely vegetated with a number of species including *Salicornia virginica*, *Frankenia grandifolia*, *Jaumea carnosa*, and *Batis maritima.* Some high marsh exists along the edges and on isolated hummocks and berms (Vogl 1966). There are extensive mud-flats with an unmuted tidal influence. UNB hosts the largest population of the Light-footed Clapper Rails in the state. Recent observations estimate the number of breeding pairs at 174, an increase over the 103 pairs observed in 1982 (Zembal, Hoffman et al. 2005).

San Diego Creek drains a highly urbanized watershed encompassing 154 square miles. The watershed, once dominated by agriculture, is now a mixture of residential, transportation, agricultural, commercial, and recreational land uses. Runoff from these land uses (both historical and current) provides a source of heavy and trace metals, organochlorine pesticides, and PCBs that are currently impairing the Bay, though the exact source, transport, and fate of each of these contaminants are unique. An investigation of selenium sources shows that shallow groundwater is a significant and constant source of selenium to surface waters in the San Diego Creek watershed. Groundwater may seep into surface waters via natural processes or it may be pumped as part of groundwater cleanup or dewatering operations and discharged into surface waters. Surface runoff is estimated to be the largest source of cadmium, copper, lead and zinc; this includes both natural and anthropogenic contributions. A recent study of pollutant inputs from tributaries within the San Diego Creek watershed concluded that the largest metals inputs come from commercial, industrial, and transportation land uses, whereas agricultural and open space exhibit the lowest loadings (Lee and Taylor 2001). DDTs, PCBs and chlordane contamination in the Bay is a result of historical use of these compounds; except for PCBs and possibly small amounts of DDT, these pollutants are no longer believed to be discharged in the watershed, except in association with erosion of sediments to which these pollutants may have adhered in the past (Masters and Iman 2000).

Sampling Design and Field Methods

The study design for this project consisted of collecting samples from three trophic levels representing the food web of the clapper rail: 1) sediments, 2) benthic macroinvertebrates, and 3) non-viable eggs of the clapper rail. Target organisms for the benthic mancroinvertebrate samples included those identified by Zembal and Fancher (1988) as the primary food source of the clapper rail and/or the primary constituents of regurgitated pellets: 1) horn snails (*Cerithidea californica),* 2) fiddler crabs (*Uca crenulata)* or lined shore crab (*Pachygrapsus crassipes*), 3) amphipods (various species), and 4) isopods (various species). Mussels (specifically ribbed horse mussels or *Ischadium demissum*) are not a primary food source but are occasionally eaten by rails (Zembal and Fancher 1988); these organisms were abundant around the nest sites and therefore also collected.

The original sampling design called for collection of 10 non-viable clapper rail eggs from active egg nests. From each nest site, a sediment sample and a set of benthic macroinvertebrates were to be collected within close vicinity to the nest. Because of limited funds initially available for the project, no replicate sediment or benthic macroinvertebrate samples were planned. Nest surveys were conducted only on Shellmaker Island and along the service road on the south side of the Bay, with the rationale that these sites are already subject to some anthropogenic disturbance and nest surveys would not likely cause a large disruption to breeding of this endangered species. During the 2003 nesting season, active nest sites were monitored and non-viable eggs screened by candling or floating. The latitude and longitude of the nest site was recorded. Single sediment and macroinvertebrate samples were collected within two weeks of egg collections within 10 m of the nest sites. Because only two eggs were collected during the 2003 season, and because additional funds were found for the project, the sampling plan was extended to 2004 and modified to sample sediments and benthic macroinvertebrates in duplicate or triplicate within a 10 m radius of the nest. Four non-viable clapper rail eggs were collected from three nest sites in 2004.

Egg samples were stored in egg cartons and refrigerated until shipped for analysis of embryonic developmental abnormalities (teratogenesis) and eggshell thickness. Once this analysis was complete, the eggs were then shipped for analysis of metal and organochlorine compounds.

Sediment samples were taken by scraping the first 0-2 cm of sediment from an area approximately 0.05 m^2. Sediments were homogenized to the extent possible in the field. A portion of the sample was stored in a Ziploc bag for grain size, and total organic carbon (TOC) analysis. Another portion destined for metal and organochlorine contaminant analysis was placed in a pre-cleaned glass jar with a Teflon-lined lid. Benthic macroinvertebrate samples were placed in a Ziploc bag and all samples stored on ice until processed in the laboratory (approximately 3-6 hours). Benthic macroinvertebrates were washed with seawater and strained to remove any excess water. They were then repackaged in clean Ziploc bags and frozen, along with the sediment samples, in a –20 ^{0}C freezer until shipped for contaminant analysis.

Figure 1 gives the locations of the nests sites for the 2003 and 2004 sampling seasons. Table 1 gives the GPS coordinates for each nest site, as well as a summary of the types of prey organisms collected. In general sites were located along the access road to Shellmaker Island or along Back

Bay Drive. Nest sites 2, 4, and 5 were all located at the transition from freshwater seeps (*Scirpus* and *Typha*-dominated) to salt marsh (*Spartina foliosa*-dominated). All sites were located well away from intertidal mudflats, so sediments and prey organisms were generally collected in areas with sparse vegetation or in small, unvegetated basins with standing water. Nest Site 5 was somewhat unique in that the area sampled around the nest was virtually barren of prey organisms. The site was located within 15 meters of a small storm drain. Only a few snails and amphipods were collected at this site.

Table 1. Summary of field sample efforts detailing date, location and number of eggs collected, and number and type of sediment and prey organisms subsampled at each nest site. S= Sediment, A= Amphipod, C= Crab, I= Isopod, M= Mussels, and Sn= Snails.

Nest Site No.	Egg No.	Date Egg Collected	Lat/Long	Habitat Type	Sediment & Prey Subsample No.	Date Collected	Type Samples Collected					
							S	A	C	I	M[2]	Sn
1	1	22-May-03	33.62155/ -117.89057	Mixed *Spartina* and *Salicornia* marsh	1	22-May-03	X	X	X	X	X	X
2	2	10-Jun-2003	33.63558/ -117.88643	*Spartina* marsh at edge of freshwater seep dominated by Scirpus and small unvegetated basins	2	10-Jun-2003	X		X	X	X	X
3	3	17-Jun-04	33.62170/ -117.89068	Mixed *Spartina* and *Salicornia* marsh	3-1	06-Jul-04	X		X		X	X
					3-2		X	X	X	X	X	X
					3-3		X	X	X	X		X
4	4A	06-Jul-04	33.63526/ -117.88620	*Spartina* marsh at edge of freshwater seep dominated by *Scirpus* and small unvegetated basins	4-1	06-Jul-04	X			X	X	X
					4-2		X	X		X	X	X
	4B	06-Jul-04			4-3		X	X		X	X	X
5	5	12-Jul-04	33.64753/ -117.88689	*Spartina* marsh at edge of freshwater seep dominated by *Scirpus* and small unvegetated basins. Close to storm drain	5-1	09-Sep-04	X	X				X
					5-2		X					

Analytical Methods

Contaminant Analysis

A total of 28 organochlorine pesticide compounds, 46 PCB congeners, and 24 heavy and trace metals were selected for analysis in sediments, prey organisms, and tissues (Table 2). In addition to contaminants, sediments were analyzed for TOC and grain size. All tissue samples were analyzed for percent lipids; tissues were not analyzed for percent moisture, which was estimated based on literature values. Analyses were performed at CRG Marine Laboratories; methods of analysis and detection limits are given in subsequent sections below.

[2] Mussels collected included the ribbed horse mussels (*Ischadium demissum*)

Table 2. List of heavy and trace metals, organochlorine pesticides and PCBs analyzed in sediments, prey organisms and clapper rail eggs.

Heavy & Trace Metals	Organochlorine Pesticides	PCBs	
Aluminum (Al)	Aldrin	Total Detectable PCBs	
Antimony (Sb)	BHC-alpha	PCB018	PCB149
Arsenic (As)	BHC-beta	PCB028	PCB151
Barium (Ba)	BHC-delta	PCB031	PCB153
Beryllium (Be)	BHC-gamma	PCB033	PCB156
Cadmium (Cd)	Total Chlordane +Chlorene +cis-Nonahlor	PCB037	PCB157
Chromium (Cr)	Chlordane-alpha	PCB044	PCB158
Cobalt (Co)	Chlordane-gamma	PCB049	PCB168+132
Copper (Cu)	Oxychlordane	PCB052	PCB169
Iron (Fe)	trans-Nonachlor	PCB066	PCB170
Lead (Pb)	Total Detectable DDTs	PCB070	PCB177
Manganese (Mn)	2,4'-DDD	PCB074	PCB180
Mercury (Hg)	2,4'-DDE	PCB077	PCB183
Molybdenum (Mo)	2,4'-DDT	PCB081	PCB187
Nickel (Ni)	4,4'-DDD	PCB087	PCB189
Selenium (Se)	4,4'-DDE	PCB095	PCB194
Silver (Ag)	4,4'-DDT	PCB097	PCB200
Strontium (Sr)	Dieldrin	PCB099	PCB201
Thallium (Tl)	Endosulfan Sulfate	PCB101	PCB206
Tin (Sn)	Endosulfan-I	PCB105	
Titanium (Ti)	Endosulfan-II	PCB110	
Vanadium (V)	Endrin	PCB114	
Zinc (Zn)	Endrin Aldehyde	PCB118	
	Endrin Ketone	PCB119	
	Heptachlor	PCB123	
	Heptachlor Epoxide	PCB126	
	Methoxychlor	PCB128+167	
	Mirex	PCB138	
	Toxaphene	PCB141	

Sediment Grain Size and Total Organic Carbon

Grain size analysis was performed using a Horiba Model LA-900 Laser Scattering Particle Size Distribution Analyzer in conjunction with Horiba Data Systems software. Data were reported as frequency (percent) of particle diameters between 0.88 and 1,000 microns. Minimum detection limit was 0.01 %.

Total organic carbon (TOC) in sediments was analyzed with a Carlo Erba 1108 CHN Elemental Analyzer equipped with an AS/23 Autosampler in conjunction with Carlo Erba Data Systems software (Castillo and Khan 1992). Frozen sediments were thawed and homogenized at room temperature, then dried at 60^{o} C overnight. An aliquot of each sample was acidified with 12N HCl vapors to remove inorganic carbon. The acidified sample was again dried, packed in a tin boat and crimped prior to CHN analysis. Acetanilide was used as the external standard. Acetanilide and cyclohexanone were used for Quality Control (QC) check standards. The certified reference material was PACS-1 (National Research Council). Minimum detection limit was 0.01 % TOC.

Heavy and Trace Metal Analysis

Analysis of heavy and trace metals was conducted for the list in Table 2. Sample preparation followed a modification of EPA Method 200.2. Approximately 0.5 g of dried and finely-ground sediment or wet soft body tissue was digested using 5 mL of hot 50% trace metal grade nitric acid and 10 mL of 25% hydrochloric acid. The supernatant with sample digest was transferred to new polyethylene bottles prior to analysis.

Inductively coupled plasma-mass spectroscopy (ICP-MS) was used to determine concentrations of all analytes in the sample digest solutions utilizing a Hewlett Packard Model 4500 with Hewlett Packard Data Systems software and following protocols established by EPA Method 6020 for tissues. The internal standard solution included scandium, gallium, rhodium, and bismuth. Major interferents included argon, sodium, and magnesium. Instrument blanks were run to identify sample carryover. The certified reference material was MESS-2 (National Research Council). Minimum detection limit in sediments and soft body tissues of prey organisms and eggs was 0.025 mg kg^{-1} for all constituents except iron and aluminum, which was 1 mg kg^{-1}. These detection limits are on a dry weight basis in sediments and on a wet weight basis for tissue.

Analysis of Organic Contaminants and Tissue Lipids

Sediment samples were thawed and homogenized with anhydrous sodium sulfate at room temperature, spiked with surrogate standards, and solvent extracted using a roller table. Extracts were then dried with anhydrous sodium sulfate, treated with copper or mercury for sulfur removal, and subjected to Florisil and/or alumina/silica gel packed columns for clean-up (SW-846, Method 3620). Each extract was concentrated and internal standards were added to the final extract prior to instrumental analysis. No samples were reported as having matrix-related interferences.

To extract lipids and organic contaminants from prey organisms and tissue, frozen samples were thawed, the soft body tissue removed from shells or carapaces (for mussels, snails and crabs) and homogenized, and the sampled weighed for extraction. Surrogate standards were added at this time. A mixture of acetonitrile, water, and hexane (2:1:2 volume to weight) was added to the tissue and homogenized with a Brinkman Polytron. The solvent layer was removed to a kilned flask after centrifugation. The process was repeated twice with hexane (1:1 v/g) added during the homogenization step. The lipid containing solvent was turbo-evaporated at 37°C, transferred to a clean 1-dram vial, and concentrated to 1 ml under nitrogen. Three microliters of extract was used to gravimetrically determine lipid concentration through evaporation. PCB extraction was done by adding concentrated sulfuric acid to the extract (1:1 v/v), mixing, centrifuging, and removing the acid layer until the hexane layer was clear. The final step before chromatographic separation was to transfer hexane to an autosampler vial and add internal standards. To determine lipid content, about 3 to 5 µL of extract was transferred using a 10-µL microsyringe to an aluminum boat placed on a microbalance. Solvent was allowed to evaporate until a constant weight was reached. The weight difference was defined as the lipid content. Minimum detection limit for lipid analysis was 0.01 % based on wet weight.

Chlorinated pesticide and PCB measurements were conducted using gas chromatographs equipped with electron capture detectors (GC-MS) (EPA Method 8270). Total chlordane was calculated as the sum of Chlordene, alpha-Chlordane, gamma-Chlordane, oxy-Chlordane, cis-Nonachlor, and trans-Nonachlor. Total detectable DDT as calculated as the sum of 2,4'-DDD, 4,4'-DDD, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, and 4,4'-DDT. Minimum detection limit for organochlorine pesticides and PCBs in sediments, prey organisms and eggs was 1 ng g^{-1} for all constituents except toxaphene, which was 10 ng g^{-1}. These detection limits are on a dry weight basis in sediments and on a wet weight basis for tissue.

Analysis of Eggshell Thinning and Embryo Teratogenesis

Each of the clapper rail eggs was opened to determine its fertility, stage of development, the position of its embryo in the egg, and whether pipping had occurred (i.e. whether the embryo had begun to break into the air cell or out of the egg). Eggs were opened by cutting the eggshell at the bottom of the aircell in order to examine the position of the embryo in the egg. The condition of the embryo and stage of embryo development at the time the egg failed was determined by visual examination. Developmental progression was determined by comparing the rail embryos to another precocial bird, the ring-necked pheasant *Phasianus cholchicus* (Hermes and Woodard 1987) and to domestic chicken developmental stages (Hamilton 1952). Embryos were examined for age and evidence of external deformities. After the egg contents were examined, the entire contents were saved in chemically cleaned containers and kept frozen until shipment to the laboratory for chemical analysis.

Eggshells were cleaned of mud, yolks, etc. using lukewarm water. Shell thickness was measured to the nearest 0.01 mm using a modified Starrett micrometer after the shells had dried at room temperature for at least one month. Five measurements of eggshell thickness were taken around the equator of the egg (Figure 2). The eggshell measurement included the shell and shell membrane. The measurements were averaged to produce a single value for each egg (Klaas, Ohlendorf et al. 1980).

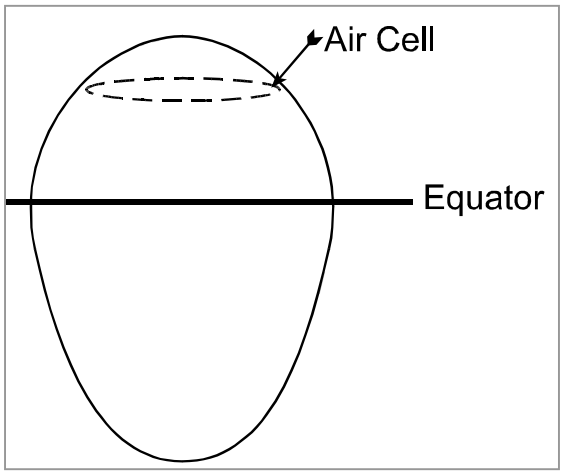

Figure 2. Approximate location of air cell and egg equator

Data Analysis

The general approach for analysis of study data was five-fold: 1) comparison of sediment contaminant concentrations with Threshold Effect Level (TEL) and Probable Effect Level (PEL) screening values for marine sediments (NOAA 1999), 2) calculation of bioaccumulation factors to determine degree of bioaccumulation and biomagnification in prey organisms and eggs, 3) comparison of prey organism contaminant concentrations with existing dietary screening values for aquatic birds, preferably the clapper rail where they exist, 4) comparison of clapper rail egg contaminant concentrations with existing screening values for biotic effects in birds, preferably aquatic birds, and 5) analysis of physical characteristics of eggs relative to contaminant chemistry to estimate the degree to which contaminants may be impacting clapper rail reproduction. References for all screening values are given in Section 3, Results. Definition of bioaccumulation and biomagnification as well as the calculation of bioaccumulation factors merits more explanation and are discussed below.

Definition of Bioaccumulation, Bioconcentration and Biomagnification

Bioaccumulation is the uptake of a contaminant from water, sediment and/or dietary sources. *Bioconcentration* is the uptake of a contaminant by aquatic organisms where water is the sole contaminant source. *Biomagnification* refers to the processes of both bioconcentration and bioaccumulation that result in increased tissue concentrations of a contaminant as it passes through two or more trophic levels (e.g., predators have greater concentrations of a particular chemical than their prey). In this study, *bioaccumulation* is used to refer to enrichment of either prey organisms or eggs relative to sediments for any particular contaminant. *Biomagnification* was used to describe enrichment of clapper rail eggs relative to prey organisms.

Calculation of Bioaccumulation Factors

A bioaccumulation factor, generally speaking, is the ratio of a substance's concentration in an organism's tissue to its concentration in the food it eats or the medium in which it lives (sediment or water). Calculation of these factors for organisms relative to sediment can be done by either: 1) bioaccumulation accumulation factor (BAF) -- a calculation of tissue concentration relative to sediment (Eqn. 1) or 2) biota to sediment accumulation factor (BSAF) – calculation of lipid-normalized tissue concentration relative to TOC-normalized sediment concentration (Eqn. 2). Both BAFs and BSAFs are unitless and, for this study, calculated on a dry weight basis for either tissue or sediment concentrations.

Eqn. 1 $$BAF_{Org\text{-}Sed} = \frac{\text{Organism Tissue Concentration (dry weight basis)}}{\text{Sediment Concentration (dry weight basis)}}$$

Eqn. 2 $$BSAF_{Org\text{-}Sed} = \frac{\text{(Organism Tissue Concentration (dry wt) / Lipid Fraction \%))}}{\text{(Sediment Concentration (dry weight) / TOC Fraction (\%))}}$$

The decision to use BAFs or BSAF for calculating for this study was based on determining whether a relationship existed between tissue contaminant concentration and lipid concentration or sediment contaminants and TOC concentrations. For both tissues and sediments, linear regressions showed no strong correlation for heavy and trace metals or organochlorine

pesticides. Because of this, we chose not to use BSAFs in calculation of bioaccumulation in egg or prey organisms relative to sediments. Instead, $BAF_{Org\text{-}Sed}$ (Eqn. 1) was used to calculate the bioaccumulation of a contaminant in clapper rail eggs or prey organisms relative to sediments. Biomagnification in clapper rail eggs relative to prey organisms $BMFs_{Egg\text{-}Org}$ was calculated using Eqn. 3. A contaminant was determined to have bioaccumulated or biomagnified in a clapper rail egg if the BAF or BMF ≥ 1.1.

Eqn. 3 $$BMF_{Egg\text{-}Org} = \frac{\text{Egg Tissue Concentration (dry weight basis)}}{\text{Prey Organism Tissue Concentration (dry weight basis)}}$$

Because percent moisture was not measured in either clapper rail eggs or prey organisms, it was necessary to use literature values to convert trace metal tissue concentrations from a wet weight to dry weight basis. 80% moisture was used to convert all prey organism tissue data to dry weight (Arnot and Gobas 2004); this resulted in a conversion factor of 5x the wet wt; 77% moisture was used to convert clapper rail egg tissue data from wet weight to dry weight (G. Santolo, unpublished data); this resulted in a conversion factor of 4.3X the wet wt.

Use of Screening Values for Sediments, Prey Organisms and Clapper Rail Eggs

Interpretation of contaminant concentrations in sediments, prey organisms and clapper rail eggs involved the use of several screening values derived from the literature. The use of these screening values and limits in their interpretation are described in the section below (C. Zeeman, personal communication).

Sediment Screening Values. Threshold Effect Levels (TELs) represent the upper limit of the range of sediment contaminant concentrations dominated by no effects data in studies compiled and reviewed by MacDonald (1994) (i.e., minimal effects range). The TEL value is a combination of both effect levels (15^{th} percentile) and no effect levels (50^{th} percentile) in the database compiled by MacDonald (1994). Concentrations below the TEL are not considered significant hazards to aquatic benthic invertebrates. Sediment numeric targets for the Toxics TMDLs are based on the NOAA TELs.

Probable Effect Levels (PELs) define the lower limit of the range of contaminant concentrations that are usually or always associated with adverse effects in studies compiled and reviewed by MacDonald (1994). The PEL is a combination of both effect concentrations (50^{th} percentile), and no effect concentrations (85^{th} percentile) in the database compiled by MacDonald (1994). Concentrations greater than the PEL are considered significant and immediate hazards to aquatic benthic invertebrates. In other words, adverse effects are <u>probable</u> when concentrations exceed the PEL. Adverse effects are considered <u>possible</u> when contaminant levels are between the TEL and the PEL.

TELs, PELs, and other similar screening levels are for directly exposed benthic invertebrates. They do not address the potential for bioaccumulation and/or potential for adverse effects in higher trophic level organisms. As a result, decisions based on TELs or similar screening values may not address risks to upper trophic level species.

Dietary Screening Levels. Dietary screening values are compared to prey organism contaminant concentrations. Those provided by C. Zeeman[3] (personal communication), given in Tables 9 and 10, are risk-based concentrations. They are derived using low-end toxicity reference values (TRV-L) derived by Region 9 EPA-BTAG (2002) and exposure factors (EPA 1993; Nagy 2001), including food ingestion rates specific to birds the size of an adult clapper rail. The TRV-L represents a chronic no observed adverse effect level (NOAEL). It is based on NOAELs identified in laboratory studies, often with adjustments for uncertainty about the duration of exposure and interspecies differences in sensitivity. The TRV-L is a daily dose rate below which no observable adverse effect is expected for the wildlife receptor of concern. The TRVs used for mercury were derived by USFWS (2003) and also represents a NOAEL for wildlife receptors.

Screening Values for Avian Reproductive Impairment
No effect levels, levels of concern and toxicity thresholds used to evaluate contaminant levels measured in clapper rail eggs are guidelines identified by the U.S. Department of the Interior (USDOI 1998) to support studies for the National Irrigation Water Quality Program in the western part of the U.S. The guidelines are defined as summary values "designed to give only a general indication of concentrations that may be troublesome in various media." The three levels vary in their meaning, depending on the contaminant. For example, the summary guidelines for mercury, selenium, and DDT are interpreted as follows.

(1) Concentrations below "no effect levels" produce no discernable adverse effects on fish or wildlife;
(2) Concentrations in the "levels of concern" range rarely produce discernable adverse effects but are elevated above typical background concentrations; median effect concentrations where the endpoint is not mortality; and
(3) Concentrations above the "toxicity threshold" value appear to produce adverse effects, such as mortality, in some fish and wildlife species.

[3] Email from C. Zeeman, Carlsbad Fish and Wildlife Office, U.S. Fish and Wildlife Service, to Steven Bay, Southern California Coastal Water Research Project.

RESULTS

Sediment and Organism Bulk Characteristics

Sediment, prey organism and clapper rail egg bulk characteristics are given in Table 3. Rail eggs, amphipods, and isopods had the highest percent lipids, with geometric means of 8.7, 2.0 and 0.8% respectively. Mussels and snails had the lowest percent lipids, with values ranging from 0.10 and 0.21% respectively. High variability in the samples existed with respect to sediment TOC and percent lipid in all prey organisms.

Table 3. Sediment, prey organism, and clapper rail egg bulk characteristics. NS = no sample; ND = non-detect where MDL for %TOC and % was 0.01%. One half the MDL was used to calculate geometric means.

Nest Site No.	Egg		Subsample No.	Sediment		Tissue % Lipid				
	Sample No.	% Lipid		% Silt and Clay	%TOC	Amphipod	Crab	Isopod	Mussel	Snails
1	1	5.20	1	56.0	3.52	0.455	0.41	0.17	0.04	0.03
2	2	9.29	2	39.0	7.48	NS	0.75	0.36	0.04	0.05
3	3	11.6	3-1	35.4	10.2	NS	0.5	NS	0.185	0.62
			3-2	75.5	4.59	11.1	1.26	1.35	0.4	0.77
			3-3	42.2	9.3	ND	0.5	25	NS	0.71
4	4A	8.05	4-1	46.6	17.51	NS	NS	1.24	0.3	0.81
			4-2	55.0	9.08	3.45	NS	1.24	0.26	0.76
	4B	7.32	4-3	55.4	9.47	1.94	NS	1.52	0.315	0.62
5	5	11.6	5-1	52.5	0.27	1.42	NS	NS	NS	0.63
			5-2	56.7	1.13	NS	NS	NS	NS	NS
Geometric Mean		8.7		49.6)	4.1	2.0	0.5)	0.8	0.10	0.21

Analysis of Heavy and Trace Metals in Sediments, Prey Organisms and Eggs

Sediment Concentrations Relative to Screening Values

Table 4 shows the concentrations of heavy and trace metals of concern for Upper Newport Bay (see also complete list of all heavy and trace metals analyzed in Appendix 1). For As, Cd, Cr, Co, Cu, Pb, Hg, Ni, Se, Ag, and Zn, a range of 8-85% of the sediment samples from the 5 egg sites had concentrations exceeding threshold effects levels (TELs). If a TEL was not available, then another screening value was used. Notably, greater than 65% of sediment samples exceeded TEL values (for benthic macroinvertebrates) for As, Cd, Cu, and Zn and the toxic effects threshold for adverse effects in some wildlife and fish species of 4 mg kg^{-1} dry wt for Se (USDOI 1998). Only Cd and Zn had concentrations in sediments that exceeded probable effects levels (PEL) in some samples.

As, Cd, Cu, Pb, and Zn concentrations found in the sediment samples showed consistent signs of anthropogenic enrichment (Table 4). 50% or greater of the samples had concentrations exceeding Fe-normalized reference levels established for southern California Bight marine sediments (Schiff and Weisberg 1999). The degree of heavy and trace metal contamination above reference

levels was fairly consistent from site to site and no clear significant spatial trends were found (Table 5).

Table 4. Summary statistics of sediment heavy and trace metal concentration relative to threshold effects level (TEL) screening values for benthic macroinvertebrates. Percentage of samples exceeding Fe-normalized background level for southern California bight marine sediments indicate degree of anthropogenic enrichment.

Element	No Detects/ Initial No. Samples	Mean (mg kg^{-1} dry wt)	StdDev	Max	Min	Threshold Effects Level (TEL)	Probable Effects Threshold (PEL)	% Samples Exceeding TEL (n=12)	% Samples Exceeding PEL or alt. Screening Value (n=12)	% Above Reference for Marine Sediments (n=13)
As	13/13	8.5	3	12.6	4.7	7.2	41.6	67	0	67
Cd	13/13	2.5	2	6.3	0.7	0.7	4.2	75	8	85
Cr	13/13	40.7	12.7	57.2	20.1	52.3	160.4	8	0	15
Co	13/13	7.3	1.6	11.6	5.2	NO TEL	10[A]	--	8[A]	N/A
Cu	13/13	38.1	13.9	67.3	11.4	18.7	108.2	75	0	77
Pb	13/13	17.4	5.2	30.2	11	30.2	112.2	8	0	62
Hg	10/13	0.08	0.02	.13	0	0.1	0.69	17	0	N/A
Ni	13/13	20.9	5.7	26.7	11.1	15.9	42.8	75	0	15
Se	13/13	4.9	1.3	8.3	3.1	1-4.0[B]	>4	--	--	N/A
Ag	9/13	0.2	0.2	0.6	0	0.7	1.77	25	0	38
Zn	13/13	168.1	59.1	299	55.4	124	271	67	9	77

[A] No TEL or PEL available. Apparent Effects Threshold used (NOAA 1999)
[B] No TEL or PEL available. Values in TEL and PEL columns represents level of concern and toxicity threshold level (respectively) for some fish and wildlife species (USDOI 1998)

Table 5. Summary of degree of contamination at each site indicating number of heavy and trace metal elements for which the sediment screening levels (TEL or surrogate) and Fe-normalized background levels were exceeded.

Site	% of Elements for which Sediment Screening Value was Exceeded (n=11)	% Elements for which Site Exceeded Fe-Normalized Reference Levels (n=8)
1	64	100
2	45	75
3-1	55	62.5
3-2	36	50
3-3	55	62.5
4-1	55	62.5
4-2	55	62.5
4-3	55	62.5
5-1	36	50
5-2	18	62.5

Evidence of Bioaccumulation and Biomagnification

Hg, Cu, Se, Zn, As, Ag and Cd all showed signs of bioaccumulation in prey items relative to sediments, with BAFs ranging from 1.2 – 183 (Table 6; for complete set of BAFs, see Appendix 1, Table A-1). However, only for Hg, Cu, and Se was there consistent bioaccumulation found at 80% or more of egg nest sites and in the majority of prey organisms collected. Of these three elements, Hg showed the highest and most consistent bioaccumulation from sediments in all prey organisms and eggs collected (Table 6). In particular, BAFs in the most important prey items (crabs, isopods and snails) where bioaccumulation was found ranged from an average of 3.85 – 15.0 relative to sediments, while the rail eggs from the five sites had an average BAF of 18.6 relative to sediment. The elevated Cu concentrations that were found in the prey organisms may have been due to the presence of the copper containing blood pigment hemocyanin, which is present in crustaceans and molluscs. Co, Ni, and Pb showed no bioaccumulation between sediments to organisms or biomagnification from organisms to eggs.

Table 6. Summary of BAF data between prey organisms and eggs relative to sediments. Y=Yes, N= No, ND=Non Detect Codes for prey organisms are as follows: M=Mussel, C=Crab, I=Isopod, S=Snail.

Element	$BAF_{Org\text{-}Sed}$					
	Bioaccumulation? (Any BAF >1.1?)	No Sites in which BAF>1.1	Total No. Prey Samples with BAF> 1.1	Range of BAFs where > 1.1	Prey Organisms found with BAFs> 1.1	Eggs with BAFs> 1.1?
Ag	Y	4	6	1.4-185	C,I,S	ND
As	Y	3	6	1.4-2.0	C,I,S	N
Cd	Y	3	4	1.3-3.0	I,S	ND
Co	N	0	-	-	-	-
Cr	Y	0	-	-	-	N
Cu	Y	5	14	1.4-8.5	C,I,S	N
Hg	Y	5	14	1.2-63	M,C,I,S	Y
Ni	N	0	-	-	-	-
Pb	N	0	-	-	-	-
Se	Y	5	8	1.5-3.3	C,I,S	Y
Zn	Y	2	2	1.2-1.3	S	N

Hg, Cu, Se, Zn, and Cr had BAFs for eggs relative to prey organisms greater than 1.1. This biomagnification was greatest in magnitude in Hg, with BMFs that ranged from a geomean of 4.5 for crabs to 6.4 for snail tissue (Table 7; for complete set of BMFs by site and sample type, see Table A-2 in Appendix 1). In the case of Cu and Zn, BAFs for eggs that exceeded 1.5 were only found with respect to mussels—a non-typical prey organism of the clapper rail. Notably, Se biomagnification between prey organisms and rail eggs was only found for mussels and snails; maximum values in these prey organisms ranged from 1.1- 1.7.

Table 7. Summary of BMFs Results for Rail Eggs Relative to Prey Organisms. Y=Yes, N= No. Codes for prey organisms are as follows: M=Mussel, C=Crab, I=Isopod, S=Snail

Element	$BMF_{Egg-Org}$				
	Biomagnification (i.e. Any BMF >1.1) ?	No Sites in which BMF>1.1	Total No. Prey Samples with BMF> 1.1	Range of BMFs	Prey Organisms found with BMFs> 1.1
Ag	N	0	-	-	-
As	N	0	-	-	-
Cd	N	0	-	-	-
Co	N	0	-	-	-
Cr	Y	2	2	1.1-2.8	M,C,I,S
Cu	Y	1	2	1.2-19	M,S
Hg	Y	5	14	1.8-13	M,C,I,S
Ni	N	0	-	-	-
Pb	N	0	-	-	-
Se	Y	2	2	1.1-1.6	M
Zn	Y	4	4	1.5-2.8	M

Heavy and Trace Metal Concentrations in Eggs and Prey Organisms Relative to Screening Values

Table 8 gives the heavy and trace metals concentrations in rail egg tissues by site. No egg was consistently high or low for all of the metals[4]. The egg from Nest Site 2 was highest for Se and Hg, while the egg from Nest Site 1 was highest for Zn, Cu, and Ni. The egg from Nest Site 3 was the highest for As. All egg samples were non-detect for Cd and Pb. Figure 3 shows the range of heavy and trace metal concentrations in rail eggs relative to that of sediment and prey organisms.

Table 8. Summary of Concentrations of Heavy and Trace Metal Egg Tissue Concentrations. All tissue concentrations given in mg kg^{-1} dry wt. ND= non-detect. A factor of 5 was multiplied by the wet wt to derived dry wts. Geometric mean and standard error were calculated assuming that ND samples are equal to ½ the detection limit of 0.025 mg kg^{-1} dry wt for all metals listed.

Compound	Egg 1	Egg 2	Egg 3	Egg 4A	Egg 4B	Egg 5	Geomean
As	0.22	ND	0.37	0.33	0.35	0.16	0.16
Cd	ND	ND	ND	ND	ND	ND	ND
Pb	ND	ND	ND	ND	ND	ND	ND
Hg	0.61	1.26	0.32	0.53	0.27	0.60	0.53
Ni	0.96	0.13	ND	ND	0.11	ND	0.05
Se	3.50	4.48	3.42	3.10	4.39	3.55	3.71
Cu	6.48	3.91	2.43	1.83	2.17	2.57	2.93
Zn	72.17	55.87	62.17	33.59	34.13	39.61	47.43

Comparison of heavy and trace metals concentration in food prey organisms versus literature dietary screening levels and biotic effect levels for aquatic birds revealed that only Hg shows some cause for concern (Tables 9 and 10).

[4] Note that statistical comparisons of egg tissue concentration not possible because of the lack of replication.

Hg concentrations in all three primary prey organisms (crabs, snails, and isopods) exceeded the risk-based screening level (0.049 mg kg^{-1} dry wt) for clapper rails, but are below measured effect level for mallards (0.5 mg kg^{-1} dry wt). The mean concentration of bird eggs (0.6 mg Hg kg^{-1} dry wt) was slightly above the "no observed effect level" of 0.4 mg kg^{-1} dry wt, but below the level of concern for black-necked stilt eggs. Only the rail egg from Nest 2 fell within this range of level of concern for reproductive effects (1.3 mg Hg kg^{-1} dry wt).

Selenium concentrations found in prey organisms (3.6-9.8 mg Se kg^{-1} dry wt) exceeded the project dietary screening values for the aquatic life (Table 9, Presser et al. 2004). Approximately 85% of prey organism samples fell within the "marginal to substantive risk" category (3 mg Se kg^{-1} dry wt) while 30% of samples fell within the substantive risk category (>7 mg Se kg^{-1} dry wt). Se in prey organisms also exceeded the risk-based dietary screening value calculated for the clapper rail of 1.6 mg Se kg^{-1} dry wt (Table 9, C. Zeeman, personal communication). Selenium concentrations in rail eggs were all below threshold for marginal risk to aquatic birds (<6 mg Se kg^{-1} dry wt; Presser et al. 2004).

The range of Zn concentrations in prey organisms and the mean concentration in eggs (21-141 and 61 mg Zn kg^{-1} dry wt respectively) were on the borderline of the range of dietary screening levels and the no effect level for aquatic birds. However, because all animals including birds have the ability to homeostatically regulate Zn in their body, these screening levels are not well quantified. Thus, no judgment can be made as to whether the Zn concentrations found in the prey organisms or rail eggs are high enough to be of concern.

The range of prey organism Cu, Pb, and Cd concentrations exceeded the projected dietary screening values for the clapper rail (16 mg Cu, 0.1 mg Pb and 0.6 mg Cd kg^{-1} dry wt tissue respectively --Table 9). However, Pb and Cd were not detected in any rail egg samples and the mean Cu concentration of 3.4 ± 1.6 mg Cu kg^{-1} dry wt found in the rail eggs was within the 'no effects threshold.'

Finally, As, and Ni concentrations in prey organisms were both below the dietary screening values for the clapper rail (Table 9). Mean As concentration in rail eggs (0.2 ± 0.1 mg As kg^{-1} dry wt) was also well below the avian "no effect" threshold for As (1.3 mg As kg^{-1} dry wt USDOI 1998). No avian biotic effect threshold was found for Ni.

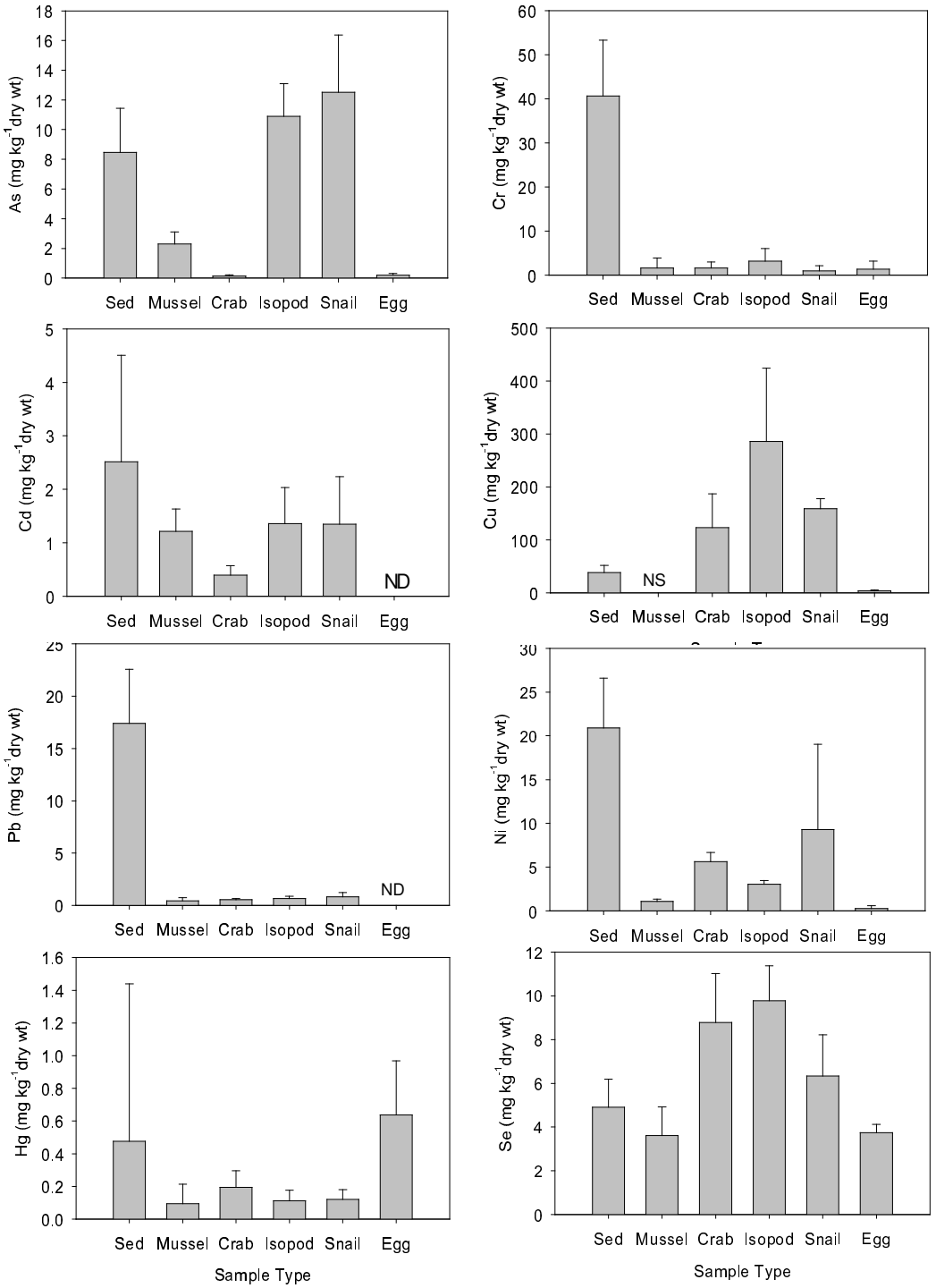

Figure 3. Geometric mean heavy and trace metal concentrations in sediments, prey organisms and clapper rail eggs for the Five Nest Sites. Error bars represent standard deviation of arithmetic mean. All values in mg kg^{-1} dry wt.

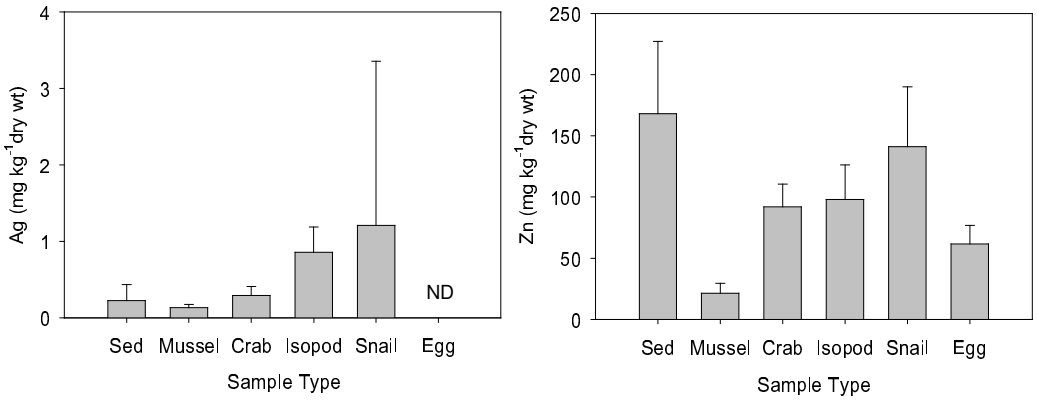

Figure 3 con't. Geometric mean heavy and trace metal concentrations in sediments, prey organisms and clapper rail eggs for the Five Nest Sites. Error bars represent standard deviation of arithmetic mean. All values in mg kg^{-1} dry wt.

Table 9 Comparison of Heavy and Trace Metals Concentrations in Prey Organisms versus Literature Dietary Screening Levels for Aquatic Birds. Explanation of derivation of risk-based screening values for clapper rail provided by C. Zeeman (personal communication) is given in Section 2.4.3.

Element	Conc. Range found in Food Prey (mg kg^{-1} dry wt)--This Study	Dietary Screening Levels for Aquatic Birds (mg kg^{-1} dry wt)	
		Level	Source
As	0.1 – 12.5	38	Clapper Rail, C. Zeeman, Personal Communication
Cd	0.4-1.4	0.6	Clapper Rail, C. Zeeman, Personal Communication
Pb	0.4-0.8	0.1	Clapper Rail, C. Zeeman, Personal Communication
Hg	0.09-0.19	0.5[A], 0.049[B]	A-Mallard reproductive effects (Heinz 1979), B -Clapper Rail, C. Zeeman, Personal Communication
Ni	1.0-9.3	9.6	Clapper Rail, C. Zeeman, Personal Communication
Se	3.6-9.8	3-7 = marginal, >7= substantive[A]; 1.6[B]	A- Presser et al., 2004 , B – C. Zeeman, Personal Communication.
Cu	122-286	16	Clapper Rail, C. Zeeman, Personal Communication
Zn	21-141	120	Clapper Rail, C. Zeeman, Personal Communication

Table 10. Summary of Trace Metal Concentrations in Eggs Versus Screening Levels for Biotic Effects on Birds. Values are in mg kg^{-1}dry wt.

Element	Rail Egg Geomean Conc. (This Study)	Biotic Effects on Birds (Based on Concentrations in Bird Eggs)			
		No effect	Level Of Concern	Toxicity Threshold	Source
As	0.2	1.3	1.3-2.8	>2.8	USDOI 1998; toxicity threshold value corrected from source to be ">2.8" rather than "<2.8"
Cd	All ND				
Pb	All ND				
Hg	0.6	0.4	0.8-4.0	3.4	A-USDOI 1998; values are converted from wet wt by factor of 5.
Ni	0.3				USDOI 1998
Se	3.7	<6	6-10	>10	Presser et al. 2004
Cu	3.5	5.5			USDOI 1998
Zn	61 (15)	50			USDOI 1998; Toxicity values not well established because all animals have ability to homeostatically regulate Zn

Analysis of Organochlorine Pesticides in Sediments, Prey Organisms and Eggs

Sediment Organochlorine Pesticide Concentrations Relative to Screening Values

Table 11 gives summary statistics for the organochlorine pesticides measured in the sediments relative to TELs and PELs for effects on benthic macroinvertebrates. The following compounds were non-detect for all sediments sampled (with minimum detection limit (MDL) of 1 ng g^{-1} for all compounds except toxaphene, which has a MDL of 10 ng g^{-1}):

Aldrin
BHC-(alpha, beta, delta, and gamma)
Dieldrin
Endosulfan (Sulfate,-I, and –II)
Endrin (and Endrin Aldehyde and Ketone)
Heptachlor and Heptachlor Epoxide
Methoxychlor
Mirex
Toxaphene

Table 11. Summary statistics of sediment organochlorine pesticide concentrations relative to threshold effects level (TEL) screening values for effects to benthic macroinvertebrates (all in (ng g^{-1} dry wt). NS = no sample; ND = non-detect where MDL = 1 ng g^{-1}. One half the MDL was used in the calculation of geometric means.

Element	No Detects/ Initial No. Samples	No. Sites For Which All Samples Non-detect	Geometric Mean	Max	Min	Threshold Effects Level (TEL)	% Samples Exceeding TEL (n=12)	Probable Effects Threshold (PEL)	% Samples Exceeding PEL Value (n=12)
2,4'-DDD	2/12	4	1.40	3.40	0.50	--	--	--	--
2,4'-DDE	1/12	4	1.25	1.25	ND	--	--	--	--
2,4'-DDT	3/12	4	1.54	5.75	ND	--	--	--	--
4,4'-DDD	4/12	3	1.73	3.75	ND	1.22	33	7.81	0
4,4'-DDE	9/12	0	7.96	42.90	3.47	2.07	75	51.7	17
4,4'-DDT	2/12	4	1.79	19.50	ND	1.19	25	4.77	0
Total DDTs	9/12	0	8.37	76.30	0.50	3.89	75	51.7	17
Chlordane-alpha	1/12	4	0.60	1.00	ND	--	--	--	--
Chlordane-gamma	2/12	4	0.80	1.98	ND	--	--	--	--
Total Chlordane	2/12	4	2.29	4.95	ND	2.26	17	4.79	17

Sediment 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, total DDT, and total chlordane exceeded TEL values for at least one site and 4,4-DDE and total DDT exceeded the TEL at all five sites. The geometric mean of total DDT and chlordane in sediments at Nest Site 5 also exceeded the PEL values (Figure 4).

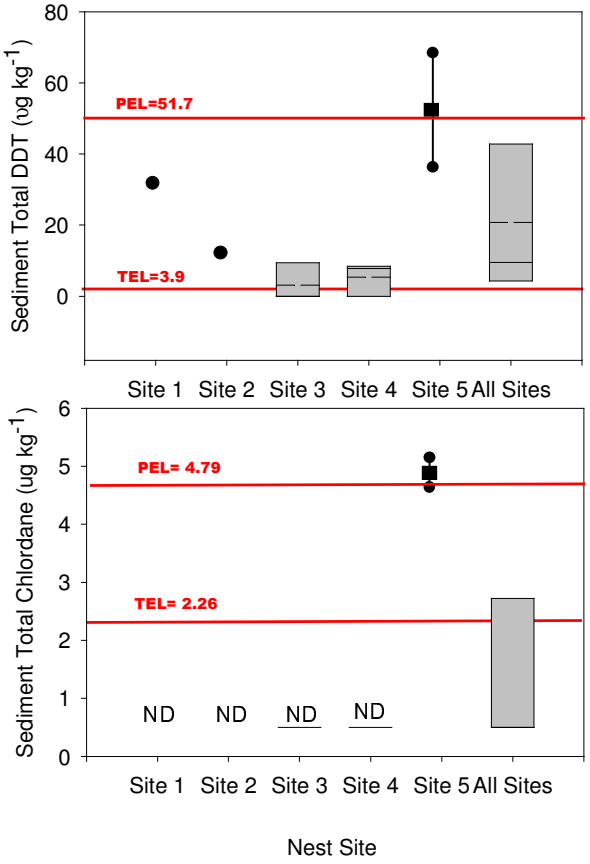

Figure 4. Mean sediment total DDT and chlordane by site relative to TEL and PEL values. Values for Sites 1 and 2 are based on one data point. Values for Site 5 are based on 2 data points so box-and-whisker plots are not shown; for this site,error bars represent maximum and minimum values. Values for Sites 3 and 4 are based on three data points; box and whisker plots for data from sites 3, 4 and all sites represent mean (solid line), median (dashed line), 25th and 75th percentiles of the contaminant.

Evidence of Bioaccumulation and Biomagnification

Of the organochlorine compounds with detectable concentrations, only 4,4'-DDE, 4,4'-DDD, and total DDT permitted calculation of bioaccumulation and biomagnification factors because these compounds were detected either in both sediments and organisms or in eggs and organisms. Chlordane (alpha and gamma), 2,4'-DDD, 2,4'-DDT, 4,4'-DDT, oxychlordane and trans-nonachlor had detectable concentrations, but only in eggs (see Section 3.3.3). The fact that some of the organochlorines were detected in eggs, but not in sediment or prey species is evidence that these compounds are present and accumulating in food web organisms, even though concentrations are too low in some compartments to calculate accumulation factors.

Substantial bioaccumulation was detected for 4,4'-DDE and total DDT from sediments to organisms at all sites (Table 12). For total DDT, maximum BAFs ranged from 2.3 –14.5 for prey organisms and 103 - 622 for rail eggs. These ranges were similar for 4,4'-DDE- the principal

constituent of total DDT found in these samples. 4,4'-DDD was only detected in sediments and egg at Nest Site 5; the BAF at this site was 25.8.

Table 12. Total DDT Biota Accumulation Factors (BAFs) for Prey Organisms and Eggs Relative to Sediment by Site. BAFs for sites 3 and 4 are the geometric means of 2-3 subsamples per site. ND= Non-detect; NS= No samples found

Element	Type	$BAF_{Sed-Org}$					
		Site 1	Site 2	Site 3	Site 4	Site 5	All Sites
Total DDT	Amphipod	ND	NS	ND	6.5	0.8	2.3
Total DDT	Mussel	0.2	ND	4.7	2.3	NS	1.2
Total DDT	Crab	1.5	14.5	4.1	NS	NS	4.5
Total DDT	Egg	131.0	220.1	621.7	518.4	102.5	248.8
Total DDT	Isopod	NS	2.2	9.7	6.3	NS	5.1
Total DDT	Snail	ND	ND	6.8	4.9	0.4	2.3
Total DDT	All Organisms	3.1	19.1	15.1	11.9	3.1	8.0

The range of BAFs detected for total DDT in rail eggs relative to sediments was higher at Nest Sites 3 and 4 (518-622; Table 12) than Sites 1, 2 and 5 (103-220). The number of prey organisms in which bioaccumulation was found was also greater at Site 3 and 4 (snails, isopods, mussels, and crabs or amphipods) than Sites 1 and 2 (crabs and mussels or isopods). Though crabs at Site 2 had the highest BAF of any prey organisms found at all sites, Sites 1 and 2 also had a greater number of non-detects in sediments or prey organisms than the other sites. At Site 5, bioaccumulation was not detected in the two types of prey organisms collected (snails and amphipods), but was found in both eggs at this site. The lack of bioaccumulation in organisms at this site is somewhat misleading, due to the almost complete lack of invertebrates found there during sampling.

No consistent trends were found in comparing BAFs among prey organisms. No single type consistently had the lowest or highest range of factors among the sites.

In general, all BMFs for total DDT in rail eggs relative to prey organisms ranged from 15 - 875, indicating biomagnification. $BMFs_{Egg-Org}$ were highest for mussels (Nest Site 1, 875) and snails (Nest Site 5, 218-- Table 13). The lowest BMF was found for crabs at Nest Site 2 (15). For other sites and organisms, BMFs were generally within the range of 50-100.

Table 13. Total DDT Biomagnification Factors (BMFs) for Eggs Relative to Prey Organisms by Site. BMFs for sites 3 and 4 are the geometric means of 2-3 subsamples per site. ND= Non-detect; NS= No samples found

Element	Type	$BAF_{Org-Egg}$					
		Site 1	Site 2	Site 3	Site 4	Site 5	All Sites
Total DDT	Amphipod	ND	NS	ND	55	100	74
Total DDT	Mussel	875	ND	87	82	NS	184
Total DDT	Crab	87	15	80	NS	NS	47
Total DDT	Isopod	NS	100	21	58	NS	50
Total DDT	Snail	ND	ND	63	68	218	98
Total DDT	All Prey Organisms	277	39	55	65	148	89

Organochlorine Pesticide Concentrations in Eggs and Prey Organisms Relative to Screening Values

Of the DDT species, only 4,4'-DDE was found in all six eggs, with concentrations ranging from 448-1050 ng g^{-1} wet wt (Table 14). Figure 6 shows the geometric mean concentrations of the DDT species in eggs relative to prey organisms.

Table 14. Summary of Concentrations of Detected Organochlorine Pesticide Residues in Eggs. All tissue concentrations given in ng g^{-1} wet wt. All other compounds analyzed were non-detect (ND) in egg samples. NA= not analyzed.

Compound	Egg 1	Egg 2	Egg 3	Egg 4A	Egg 4B	Egg 5	Geomean
4,4'-DDD	ND	ND	ND	ND	9.2	19.3	4.2
4,4'-DDE	654.0	565.0	448.0	690.0	582.0	1050.0	643.3
4,4-DDT	ND	ND	ND	62.5	ND	ND	4.2
Total DDT	654.0	565.0	448.0	702.5	590.0	1066.8	647.4
Oxychlordane	NA	NA	ND	74.8	34.9	99.4	13.6
Trans-nonachlor	ND	ND	ND	13.2	15.9	31.1	2.2

Trans-nonachlor was found in eggs from Nests sites 4 and 5, with concentrations ranging from 13-31 ng g^{-1} wet wt (Table 14). Oxychlordane, which was only analyzed in 2004 samples (Nests Sites 3-5), was found in Nest Sites 4-5 at a range of 34-99 ng g^{-1} wet wt.

The range of 4,4'-DDE concentration in the prey organisms (0.75 - 32 ng g^{-1} wet wt) was well below the dietary screening values that represent levels of concern for insectivore waterfowl of 200-600 ng g^{-1} wet wt (converted from dry wt values; USDOI 1998). However, the upper range of 4,4'-DDE concentration measured in prey organisms exceeded the risk-based screening level of 17 ng g^{-1} wet weight for DDT and metabolites (derived from a TRV for reproductive effects in pelicans; C. Zeeman, personal communication).

The minimum value as well as the geometric mean of 4,4'-DDE in the 6 clapper rail eggs (448 and 643 ng g^{-1} wet wt respectively) exceeded the lower range of the threshold for concern for omnivore waterfowl (250 – 20,000 ng g^{-1} wet wt; USDOI 1998). No avian screening values were found for oxylchlordane, trans-nonachlor, or total chlordanes.

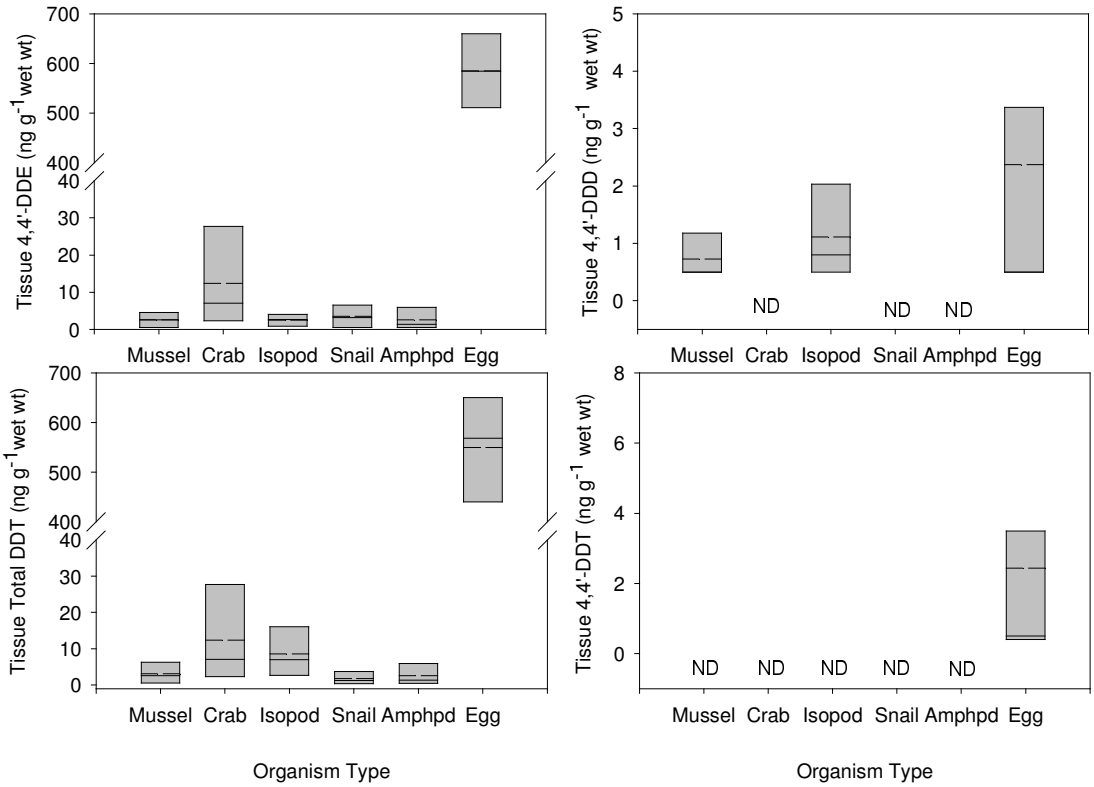

Figure 5. Box and whisker plots showing mean (solid line), median (dashed line), 25th and 75th percentiles of DDT species concentration in eggs relative to prey organisms for all site combined. ND= non-detect; Amphpd= Amphipod. All values in ng g^{-1} wet wt tissue.

Analysis PCBs in Sediments, Prey Organisms and Eggs

Sediment PCB Concentrations

Sediment samples from the five sites were non-detect for the 46 PCB congeners analyzed. Table 1 gives a list of these congeners. Minimum detection limit for these compounds is 1 ng g^{-1}. Because PCBs were below the limits of detection in sediments, it is not possible to assess risk posed by total PCBs to benthic macroinvertabrates, although it is probably low. The TEL for total PCBs (2.2 ng g^{-1} dry weight) is only an order of magnitude higher than the detection levels for individual congeners.

Evidence of PCB Bioaccumulation and Biomagnification

All prey organism samples as well as sediment samples from the five nest sites were non-detect for the 43 PCB congeners analyzed. Therefore, no calculation of bioaccumulation or

biomagnification was possible for these samples. In addition, it is not possible to compare PCB concentrations in prey organism tissues to dietary screening levels that exist for total PCBs and a few PCB congeners.

PCB Concentrations in Eggs Relative to Screening Values

Five of the six clapper rail eggs had detectable concentrations of nine PCB congeners (Table 14). Only the egg from Nest Site 2 was non-detect for all 43 congeners analyzed. A review of literature shows that the maximum concentration of total PCBs found in rail eggs (126.8 ng g^{-1} wet wt) was far below levels of concern for concentrations that produced decreased hatching success for terns and cormorants (>5000 ng g-1 wet wt, Hoffman, Rice et al. 1996)

Table 15. Summary of Concentrations of Detected PCB congeners by Egg. All tissue concentrations given in ng g^{-1} wet wt. All other compounds analyzed were non-detect (ND) in egg samples. Geometric means were calculated assuming that ND samples were ½ of detection limit (0.5 ng g^{-1} wet wt).

Compound	Egg 1	Egg 2	Egg 3	Egg 4A	Egg 4B	Egg 5	Geomean
PCB 105	ND	ND	5.7	ND	ND	8.5	7.0
PCB 118	13.3	ND	47.0	13.5	23.5	12.6	19.4
PCB 128+167	9.7	ND	ND	ND	ND	ND	9.7
PCB 138	16.3	ND	11.1	16.4	29.0	20.5	16.9
PCB153	35.2	ND	24.9	29.3	43.3	33.7	32.0
PCB 158	12.2	ND	ND	ND	ND	ND	12.2
PCB 170	ND	ND	ND	ND	ND	4.6	4.6
PCB 180	13.6	ND	11.4	10.8	21.1	18.1	14.3
PCB187	10.3	ND	7.4	ND	9.9	7.6	8.7
Total PCBs	110.6	ND	71.3	70.0	126.8	105.6	94.1

Analysis of Embryo Teratogenesis and Egg Shell Thinning

Results of Embryo Examination

Clapper rail eggs from Nest Sites 1-4 showed no evidence of developmental abnormality. The contents of the egg from Nest Site 1 were addled and no embryo was found indicating that little or no embryo development occurred before the egg failed. Due to the condition of the egg contents, it could not be determined if the egg was fertile or infertile. The egg from Nest Site 2 contained an embryo. Based on the condition of the beak, legs, feet, and position of the embryo in the egg, the embryo was full term (approx. 20 days) and was in the process of pipping (breaking out of the shell) when it died. The embryo/chick was badly decomposed and the egg was filled with maggots. It was difficult to determine definitively the condition of the embryo (normal or abnormal) due to the advanced state of decomposition. However, the eyes, beak, legs, feet, and wings appeared normal. The contents of Nest Egg 3 appeared normal for a Day 1 (Stage 6) embryo. The intermediate streak and area pelucida were visible (Figure 6a). The contents of Egg 4A (Nest Site 4) were addled, rotting, and decomposing. No embryo was found indicating that little or no embryo development occurred before the egg failed. Due to the condition of the

egg contents, fertility could not be determined. Egg 4B contained an embryo. Based on the condition of the eyes, beak, legs, feet, and position of the embryo in the egg, the embryo was about Day 12 of incubation (approx. Stage 37). The embryo/chick was beginning to decompose. The eyes, beak, legs, feet, and wings and this embryo appeared to be normal. The egg tooth was visible on the upper mandible (Figure 6b).

Of the six clapper rail eggs examined, only the egg from Nest Site 5 was found to have developmental abnormalities. This egg contained an embryo approximately Day 14 to 15 (Stage 38). The egg was cracked, the contents were rotting, and the embryo was decomposing; however, its condition was good enough that it could be evaluated. Gross examination of this embryo identified an abnormally shaped upper mandible (i.e., squared off) and a missing lower mandible, and curled toes (Figure 6c). Eyes and feathering appeared normal for an embryo in this stage of decomposition.

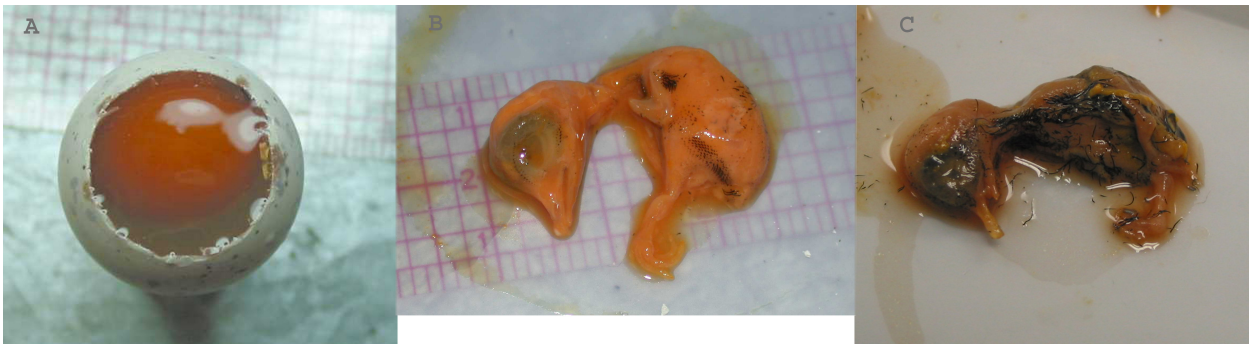

Figure 6. Picture of (a) Day 1 normal embryo from Nest Site 3, (b) a normal Day 12 embryo from Nest Site 4B, and (c) an abnormal Day 14-15 embryo from Nest Site 5.

Eggshell Thickness

Table 15 gives the results of the measurement of eggshell thickness in the six clapper rail eggs. Because the contents of the egg from Nest Site 1 were sun-baked and coagulated, an accurate measurement of the eggshell could not be made; therefore, this measurement was not included in comparisons. The mean shell thickness of the five eggs from Nest Sites 2-5 was 0.255 mm. The egg from Nest Site 5 had the lowest eggshell thickness measured (0.239 mm). An analysis of the correlation between 4,4'-DDE concentration and eggshell thickness indicated a significant inverse relationship (R^2=0.68, p-value$_{\alpha=0.1}$ = 0.042; see Figure 7).

Table 16. Eggshell thickness of non-viable clapper rail eggs from Nest Sites 1-5, with mean and standard deviation for Nest Sites 2-5. Result from each egg based on mean of three to five measurements. Results from egg from Nest Site 1 not included in mean because an accurate measurement was not possible (see text for explanation).

Nest Site	Egg Shell Thickness
1	--
2	0.267
3	0.255
4A	0.256
4B	0.260
5	0.239
Mean (Std Dev)	0.255(0.010)

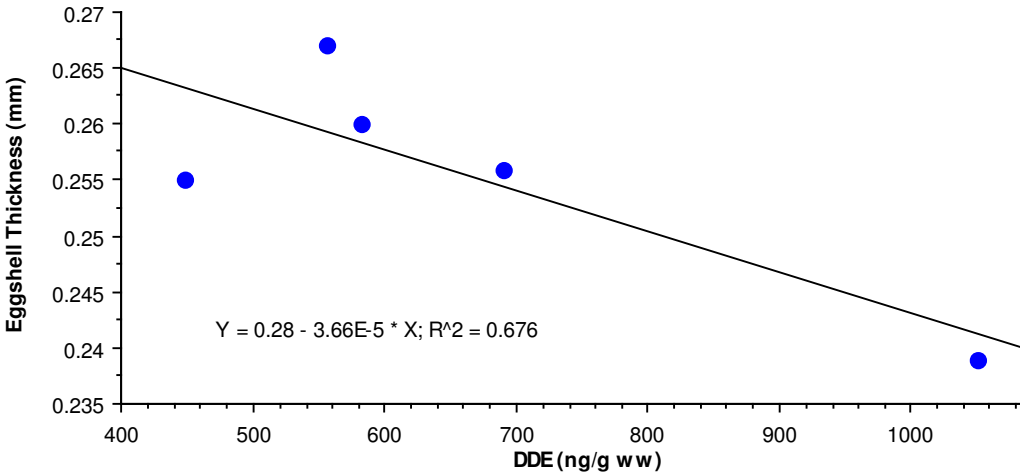

Figure 7. Linear regression between UNB clapper rail eggshell thickness and 4,4'-DDE concentration.

DISCUSSION

Contamination of avian food webs with organochlorine pesticides, PCBs, and heavy and trace metals such as selenium and mercury is a global problem (Forenback 1972, Eisler 1985, Eisler 1986, Eisler 1990). The tendency of these contaminants to persist in the environment and biomagnify has led to both acute and chronic effects in birds. Estuarine birds are particularly vulnerable because 1) estuaries are the ultimate drainage basins of urban and agricultural runoff from watersheds—thus making them potential contaminant "hot spots" and 2) they are already stressed by habitat loss, fragmentation, excessive predation, and other anthropogenic impacts. Reproductive impacts found for this suite of contaminants are especially problematic for threatened and endangered species, because populations of species that have limited genetic pools are more vulnerable to these types of impacts.

This study found that DDT and its metabolic products, technical chlordane compounds, PCBs, selenium, and mercury are present and biomagnifying in the food web of the Light-footed Clapper Rail in Upper Newport Bay. Of these compounds, 4,4'-DDE is the contaminant of greatest concern. The rationale for this finding lies in 1) DDE concentrations exceed screening levels for sediments and bird eggs and 2) embryonic abnormalities and eggshell thinning occur in the clapper rail egg that contains elevated DDE concentrations. However, we also note that because egg contaminant concentrations may be biased upward somewhat as a result of the exclusive use of eggs which failed to hatch, this study presents a "worst-case scenario." Because of this issue, along with the limited sample size and extent of spatial sampling in the estuary, it is important to recognize that this is a screening level study to determine whether cause for concern is merited and to recommend if further study is required. Levels of Se and Hg in sediments and prey organisms are within a range of concern for aquatic life and for risk-based dietary screening levels for clapper rails, but the concentrations of these contaminants in clapper rail eggs were below screening levels considered likely to impair reproduction. Because of the limited number of egg samples analyzed, it is recommended that additional data be collected to test the hypothesis that DDT or Se may be causing ecologically-significant effects to rail reproduction.

Organochlorine Compounds

DDT. DDT was an organochlorine pesticide that was widely used for agricultural purposes. Although DDT was an effective pesticide, the EPA banned its use in 1972 because of its negative ecological impacts (Hauge, Bukowski et al. 1990). Once in the environment, DDT degrades into DDE and DDD. Banned from use in California in 1970, DDT and its metabolites still persist in Upper Newport Bay. A recent study of fish in Upper Newport Bay found that DDT is present at levels of concern in commercial and forage fish (Allen et al. 2004). DDT may affect clapper rails several different ways. It may be directly toxic to adults that consume contaminated prey (e.g., reduced survival), it may adversely affect offspring (reproductive effects, such as embryotoxicty, teratogenesis, eggshell thinning) and it may have indirect effects by reducing productivity of invertebrate food sources for rails (Goodbred, Ledig et al. 1996). Biomagnification of DDT from sediments through prey organisms to the predators makes contamination of estuaries by this contaminant particularly problematic. Maximum BMFs of 126-874 for total DDT in UNB rail eggs relative to individual prey organisms were higher than found in a similar study of Light-footed Clapper Rail eggs relative to crabs in Mugu Lagoon and Tijuana Slough (BAFs= 25-63; Goodbred, Ledig et al. 1996).

Despite the biomagnification of DDT and its metabolites through the food web of the UNB clapper rail, DDT concentrations in prey organisms (0.75 – 32 ng g^{-1} wet wt) were well below levels that have been shown to be acutely toxic to Northern Clapper Rails feeding on them (LC_{50} of 3.2- 3.8 x10^5 ng g^{-1} wet wt) (Van Velzen and Kretzer 1975). Van Velzen and Kretzer's data (1975) represent median lethal levels for five days of exposure only; lower concentrations may be lethal under chronic exposure conditions. However, given the fact that the LC50 is 3-6 orders of magnitude higher than the range of DDT found in prey concentrations, it suggests that no avian mortality is occurring in Upper Newport Bay as a result of DDT contamination.

Chronic impacts, such as reproductive impairment, can occur at levels several orders of magnitude below acute toxicity thresholds. DDT concentrations in some Upper Newport Bay samples exceed a risk-based dietary screening level for clapper rails (17 ng g^{-1} wet weight) computed from the EPA (1995) NOAEL for pelicans (C. Zeeman, personal communication). However, DDT concentrations measured in the clapper rail prey organisms are below lowest observed adverse effect levels (LOAELs) for significant reproductive impairment in studies reviewed by EPA (1995), including the LOAEL for pelicans, which appear to be the most sensitive of avian species (150 ng g^{-1} wet weight).

Tolerance to DDE toxicity can vary widely between species. Because egg screening values are not available for clapper rail eggs per se, screening values for other species must be used, albeit with caution. Comparisons were made using screening values from other omnivorous aquatic birds when available. The maximum DDE concentration found in the egg from Nest Site 5 (1050 ng g^{-1} wet wt) is below that which caused reduced clutch size, decreased productivity and increased incidence of shell cracking in the white faced ibis (3000 ng g^{-1} wet wt; Henny et al. 1985); but 4,4'-DDE was found in all 6 clapper eggs at concentrations exceeding levels of the lower range of the level of concern for biotic effects in omnivore waterfowl (250 ng g^{-1} wet wt, USDOI 1998). This suggests that DDT could be causing some degree of reproductive impairment of clapper rails in Upper Newport Bay.

Goodbred, Ledig et al. (1996) measured organochlorine contaminant concentrations in the Light-footed Clapper Rail in Mugu Lagoon, Seal Beach and Tijuana Slough salt marshes in southern California. Rail egg total DDT concentrations from Upper Newport Bay are within the same range of concentrations found in Tijuana Slough but lower than those found in Mugu Lagoon – the site of the heaviest DDT contamination (Table 16). Total DDT concentrations in Mugu Lagoon rail eggs, collected in 1989-1990 were associated with an egg failure rate of 8.5 % and a reduced hatching success rate of 50% (Ledig 1990), though this reproductive impairment could be attributed to factors such as low genetic variation or predation in addition to contaminant effects. The geometric mean of total DDT in UNB rail eggs from this study is an order of magnitude lower than that of Mugu Lagoon in the 1980s. Notably, it is also almost an order of magnitude less than total DDT concentrations measured in UNB in 1983, in which the maximum DDT concentration found in a rail egg in UNB was the highest ever reported for this species (10,800 ng g^{-1} wet wt; Table 20; Ohlendorf, unpublished data). While there may be some reproductive impairment occurring in UNB presently, this declining trend in clapper rail egg DDT concentration and reports of a near doubling in the number of nesting pairs from 103 in

1979-1981 to 174 in 2004 (Zembal, Hoffman et al. 2005) suggest that impacts of stressors such as DDT on UNB clapper rail reproduction may be diminishing over time.

Table 17. Comparison of organochlorine contaminant concentrations in Light-footed and California Clapper Rail eggs from UNB and other salt marsh sites in southern and northern California. All values are in ng g^{-1} wet wt.

Site	Clapper Rail Subspecies	Date Eggs Collected	Source	Geometric Mean & (Range)			
				Total DDT	Trans-nonachlor	Oxychlordane	Total PCBs
UNB (n=6)	*R.l. levipes*	2003-4	This Study	647 (448-1066)	2 (ND-31)	14 (ND-99)	94 (74-126)
UNB (n=7)	*R.l. levipes*	1983	Ohlendorf, unpublished data	2202 (340-10,800)	225 (97-380)	151 (82-250)	934 (660-1400)
Seal Beach (n=9)	*R.l. levipes*	1989-91	Goodbred et al. 1996	460 (197-964)	30 (7-64)	64 (41-115)	316 (56-895)
Mugu Lagoon (n=19)				2230 (640-5740)	60 (17-114	119 (60-366)	44 (ND-1590)
Tijuana Slough (n=12)				1030 (60-1800)	12 (7-29)	61 (98-397)	127 (39-319)
S. San Francisco Bay (n-13)	*R.l. obsoletus*	1986	Lonzarich et al. 1992	340	120	30	820
N. San Francisco Bay (n=7)		1986-87		400	110	100	810

Physical examination of the eggshell and embryos suggests that 4,4'-DDE could be contributing to the eggshell thinning and developmental abnormalities observed in the egg from Nest Site 5. Eggshell thinning has been correlated to DDE residues in a variety of bird species (Furness 1993), although not all studies have found this correlation (King, Custer et al. 1991; Schwarzbach, Henderson et al. 2001). In this study, a trend was found between eggshell thinning and DDE residue (r=- 0.86, p-value = 0.08). Goodbred, Ledig et al. (1996) also found a correlation between DDE concentration and eggshell thickness (r = -0.43, p-value=0.035), and noted that DDE concentration only explained 19% of the variation in eggshell thinning – suggesting other factors are involved as well. Mean UNB eggshell thickness (0.255 ± 0.01 mm and range of 0.239- 0.267 mm) was similar to the mean of pre-DDT era (<1947) eggshell thickness measured from 80 eggs in the collection of the Western Foundation of Vertebrate Zoology, Camarillo California (ranged from 0.234 – 0.272 mm with a mean of 0.251 mm; Goodbred, Ledig et al. 1996). Therefore, the thinning found in the egg from Nest Site 5 may not be biologically significant at the population level. However, evidence of thinning even at the individual level is important when dealing with endangered species.

The cause of failure of the six clapper rail eggs, and in particular the developmental abnormality observed in Nest Egg 5 could not be absolutely determined from the gross embryo examinations. Eggs fail for many reasons including nest flooding, nest abandonment, and extreme weather conditions. Developmental abnormalities can be the result of a variety of factors including climatic factors, nutritional constraints, single mutations, inbreeding, or actions of various compounds that have teratogenic effects on embryos. The expressions of developmental toxicity include embryo death, malposition, malformation, growth retardation, and functional disorders. As the exposure to teratogenic compounds increases, frequency and severity of these outcomes

may increase (Hoffman, Smith et al. 1993). Significant correlation between certain malformations and contaminant levels has been identified in the avian embryo (Romanoff and Romanoff 1972). The types of abnormalities can sometimes provide insight to identify the potential teratogenic agents (e.g., Landauer 1967, Romanoff and Romanoff 1972).

Of the rail eggs salvaged from Upper Newport Bay in 2003 and 2004, Egg 5 contained the only abnormally developing embryo; it was also the egg with the highest DDE concentration (1050 ng g^{-1} wet wt.) and thinnest eggshell of the rail eggs salvaged. While the state of decomposition and the fragility of the embryo in Egg 5 made the diagnosis difficult, the abnormalities observed in the Egg 5 embryo were not characteristic of mechanical or climatic effects or of a specific contaminant. (JM Pisenti, Ph.D., UC Davis Dept. of Animal Science, personal communication) Although organochlorines are known to cause embryo mortality and abnormalities, there is no suite of abnormalities that are characteristic of organochlorine toxicity. However, in the Great Lakes, where elevated levels of persistent organochlorine contaminants (including DDEs and PCBs) were responsible for deformities in aquatic bird species, the most frequently observed deformity was that of the bill, including missing jaws (Fox 2001).

The DDT concentrations found in the clapper rail eggs and the eggshell thinning and development abnormality observed in the Egg from Nest Site 5 gives cause for concern that DDT may be impairing reproduction of the clapper rail in UNB. However, the focus of the study on failed eggs rather than a random selection of viable and non-viable eggs, the limited sample size, and the lack of greater spatial sampling in the Bay limits the degree to which any definitive conclusions can be drawn from this study. Some tendency to err on the side of caution is warranted, because thresholds specific for clapper rails are not available and the species is endangered, making the overall population sensitive to effects of multiple contaminants on individuals. Further study is recommended to investigate the degree of reproductive impairment by looking at DDT levels in randomly-selected viable eggs throughout the estuary as well as following the hatching success rate of Clapper Rail nests. Notably, clapper rails feed at a lower trophic level than shorebirds. Consequently, the results of this study should be considered in conjunction with results of other studies that provide data for higher trophic level feeders when developing TMDLs for the San Diego Creek watershed. Such a study is currently being conducted through SCCWRP in lower San Diego Creek and Upper Newport Bay.

PCBs and Chlordane. Based on this screening level study, the lack of PCBs in sediments, prey organisms, and low levels in rail eggs indicates that PCBs are not likely to be off concern in the food web of the UNB clapper rail population, though they do appear to be biomagnifying in clapper rail eggs. Mean and maximum level of total PCBs found in UNB clapper rail eggs (94 ng g^{-1} and 126.8 ng g^{-1} wet wt, respectively) were an order of magnitude below levels thought to be of concern for decreased hatching success for Aroclor 1242 in chickens – the most sensitive bird species to PCBs found to date (870 ng g-1 wet wt, Britton and Huston; Schwarzbach, Henderson et al. 2001). Aroclor 1242, one of the most toxic combinations of PCB congeners, was non-detect in all UNB rail eggs. For PCB congeners mixtures such as Aroclor 1254 and 1260 (also non-detect in UNB rail eggs), the threshold for reproductive impairments is five-fold higher (Plantonow and Reinhart 1973; Schwarzbach, Henderson et al. 2001). Total PCB residues in UNB rail eggs were 4 fold lower than those found in Mugu Lagoon- the most contaminated salt

marsh site for which data are available in southern California (Goodbred, Ledig et al. 1996) and an order of magnitude lower than values found for *Rallus longirostris obsoletus* in San Francisco Bay (Lonzarich, Harvey et al. 1992; Schwarzbach, Henderson et al. 2001). Allen et al. (2004) noted that PCB residues in forage fish in the Bay were above recommended screening values, but that these fish came primarily from lower Newport Bay. They also noted that PCB residues in fish have declined over the last two decades. As with DDT, it is important to consider that because of the limited sample size and spatial sampling, further study is recommended to definitively determine the extent of PCB contamination in the UNB clapper rail food web.

Reproductive effects of chlordane in avian eggs are not expected at concentrations less than 100 ng g^{-1} wet wt (Schwarzbach, Henderson et al. 2001). Henny, Blus et al. (1983) found reproductive impairment in the American Kestrel (*Falco sparverius*) when eggs concentrations of oxychlordane exceeded 1500 ng g^{-1} wet wt. Because mean values of total chlordane in the study did not exceed 30 ng g^{-1} wet wt, we would not expect acute or chronic effects from chlordane in UNB. In addition, Table 22 illustrates that chlordane concentrations in UNB rail eggs are lower than those found in other salt marsh sites in southern California (total chlordane of 73-179 ng g^{-1} wet wt; Goodbred, Ledig et al. 1996) and San Francisco Bay (90 – 210 ng g^{-1} wet wt; Schwarzbach et al. 2001, Lonzarich et al. 1992).

It is worthwhile to note that the concern about all of the contaminants that bioaccumulate is that conclusions about exposure, risk and impacts on clapper rails may not be applicable for species that occupy higher trophic levels. Consequently, while results of this study support a screening level assessment of contaminant-related risk to clapper rails in Upper Newport Bay, risks to higher trophic level receptors will have to be factored in when TMDLs are developed.

Trace Elements, Metals, and Metalloids

Selenium. At elevated dietary levels, Se can results in acute toxic effects as well as chronic effects like the impairment of reproduction of aquatic birds (Eisler 1985; Heinz, Hoffman et al. 1987; Ohlendorf, Hothem et al. 1989). Se data from this study showed that some cause for concern is merited. Greater than 65% of sediment samples from the five nest sites exceeded toxicity effects levels of 4 mg kg^{-1} dry wt for some fish and wildlife species (USDOI 1998). Selenium concentrations found in some prey organism (3.6-9.8 mg Se kg^{-1} dry wt) exceeded projected dietary screening values for aquatic birds (3-8 mg kg^{-1} dry wt; NIQWA 1998) and also exceeded the risk-based dietary screening value calculated for the clapper rail of 1.6 mg Se kg^{-1} dry wt (Table 9, C. Zeeman, personal communication). Some bioaccumulation was also observed to be occurring in rails relative to prey organisms (BMFs of 1.1- 1.7) and in prey organisms relative to sediment (BAFs of 1.5- 3.3).

Rail egg concentrations were elevated relative to concentrations founds in non-viable clapper rail eggs in Seal Beach and Tijuana Slough, where all egg samples (n=8) were non-detect for selenium (Hui, Goodbred et al. 2002). Despite the fact that UNB rail egg Se concentrations were elevated relative to those in other southern Californian salt marshes, selenium concentrations in UNB rail eggs (geometric mean of 3.7 mg kg^{-1} dry wt and range of 3.1-4.5 mg kg^{-1} dry wt) were below the threshold of marginal risk to aquatic birds (<6 mg kg^{-1} dry wt; Presser et al. 2004). The marginal risk level for eggs of 6 mg kg^{-1} dry wt is based on individual response thresholds (e.g., Heinz 1996; Skorupa 1998). Extensive study of Se in aquatic birds at Kesterson

Reservoir has allowed good documentation of the characteristic signs of Se-induced teratogenesis including reduced or missing eye in the embryo (Ohlendorf, Hothem et al. 1990; Skorupa and Ohlendorf 1991). The lack of these characteristic deformities observed in the egg from Nest Site 5 makes selenium toxicity a less likely cause for the developmental abnormality observed. In addition, a combination of effects from DDE, Se, or other contaminants could also be responsible, but it is not possible to definitely determine the cause of the deformity in this egg.

Allen et al. (2004) found that forage fish in UNB had Se levels that exceeded wildlife screening values. They noted that Se concentrations tended to be elevated in species that lived at or near the edge of the marsh. Since the nest sites sampled were on the edge of UNB and away from the primary source of Se (San Diego Creek), a more extensive study of Se in the food web of clapper rails as well as fish-eating birds is recommended in order to determine more conclusively whether Se may be impairing reproduction in UNB birds. A study of Se and organochlorine contamination in the food webs of shorebirds and waterfowl found nesting in UNB and San Diego Creek is ongoing and will provide information on the degree of reproductive impairment in these species.

Mercury and Other Trace Elements, Metals, and Metalloids. Of other trace elements, metals, and metalloids, Hg was the only contaminant whose concentrations were sufficiently elevated in UNB sediments, prey organisms and rail eggs to be of concern. Hg, as well as several other elements (Co, Cu, Pb, Ni, Ag and Zn), had sediment concentrations exceeding TEL values (NOAA 1999). Only Hg, however, exceeded dietary screening levels (0.045 mg Hg kg^{-1} dry wt) in three of the primary prey organisms of the clapper rail (crabs, isopods, and snails; 0.15-0.2 mg Hg kg^{-1} dry wt). Biomagnification of Hg was also most significant of all trace elements and metals measured (BAFs of 4-6). Hg has been shown to have a synergistic effect on embryo terratogenesis and mortality with Se (Hoffmann and Heinz 1998).

Mean Hg concentration in UNB clapper rail eggs (0.64 mg kg^{-1} dry wt) exceeded the no effect level (0.43 mg kg^{-1} dry wt). However, only in the egg from Nest Site 2 (1.3 mg kg^{-1} dry wt) was the concentration within the lower range of the level of concern (0.8 – 4.0 mg kg^{-1} dry wt; USDOI 1998). Based on these results, it is unlikely that Hg is significantly impairing clapper rail reproduction in UNB. However, because it is present at levels of concern in various media to which clapper rails are exposed, Hg should be a contaminant to further monitor in UNB. Hg, like other contaminants that can biomagnify, may be impacting species that occupy higher trophic levels than that of the clapper rails.

CONCLUSIONS

This study found that DDT and its metabolic products, chlordane compounds, selenium, and mercury are present and biomagnifying in the food web of the Light-footed Clapper Rail in Upper Newport Bay. Of these compounds, 4,4'-DDE is the contaminant of greatest concern. The rationale for this finding lies in 1) DDE concentrations in one egg exceeding screening levels for birds and 2) examination of embryonic abnormalities and eggshell thinning support the case that DDE may be, to a limited extent, impairing reproduction of the clapper rail. Levels of Se and Hg in sediments and prey organisms are within a range of concern, although the concentrations of these contaminants in clapper rail eggs were below levels considered to significantly impair reproduction. Because of the exclusive use of non-viable eggs, these results present a "worst case scenario" appropriate for a screening level study. Because of this issue, along with the limited sample size and extent of spatial sampling in the estuary, we recommend further study to determine the extent to which DDT, selenium, and mercury may be currently impairing reproduction of the clapper rail in Upper Newport Bay. Such a study would complement ongoing studies looking at metals and organochlorine contamination in the food webs of UNB fish and other wading and pisciverous birds.

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APPENDICES

Bioaccumulation and Biomagnification Factors by Site and Sample Type

Table A-1. Bioaccumulation factors (BAF) for prey organisms and eggs relative to sediments. BAFs for sites 3 and 4 are the geometric means of 2-3 subsamples per site. NS= No sample, ND= non-detect.

Analyte	Type	Site 1	Site 2	Site 3		Site 4		Site 5
		$BAF_{Sed-Org}$	$BAF_{Sed-Org}$	$BAF_{Sed-Org}$	Stdev	$BAF_{Sed-Org}$	Stdev	$BAF_{Sed-Org}$
As	Mussel	0.29	0.39	0.17	0.03	0.17	0.00	NS
As	Crab	0.94	1.99	1.18	0.62	NS	NS	NS
As	Egg	0.02	ND	0.04		0.02	0.01	0.06
As	Isopod	0.77	1.44	1.30		1.14	0.25	NS
As	Snail	0.76	1.63	1.80	0.40	0.62	0.48	2.03
Cd	Mussel	1.03	0.29	0.99	1.32	0.50	0.05	NS
Cd	Crab	0.49	0.07	0.02	0.03	NS	NS	NS
Cd	Egg	ND	ND	ND		ND	0.00	ND
Cd	Isopod	2.06	0.24	1.76		0.39	0.17	NS
Cd	Snail	0.64	0.21	1.33	1.05	0.48	0.28	3.04
Co	Mussel	0.04	0.09	ND	0.00	0.02	0.00	NS
Co	Crab	0.14	0.14	0.09	0.06	NS	NS	NS
Co	Egg	ND	ND	ND		ND	0.00	ND
Co	Isopod	0.06	0.15	0.07		0.08	0.01	NS
Co	Snail	0.10	0.09	0.21	0.04	0.05	0.06	0.14
Cr	Mussel	0.02	0.11	ND	0.00	ND	0.00	NS
Cr	Crab	0.03	0.07	ND	0.00	NS	NS	NS
Cr	Egg	0.03	0.10	ND		ND	0.00	ND
Cr	Isopod	0.03	0.07	0.18		ND	0.00	NS
Cr	Snail	0.05	0.03	ND	0.00	ND	0.00	ND
Cu	Mussel	0.81	0.23	0.43	0.13	0.14	0.01	NS
Cu	Crab	4.65	3.47	0.44	0.48	NS	NS	NS
Cu	Egg	0.17	0.09	0.05		0.05	0.01	0.18
Cu	Isopod	5.73	4.47	11.82		4.12	1.00	NS
Cu	Snail	4.06	3.53	3.65	0.61	1.42	1.59	8.49
Pb	Mussel	0.02	0.04	ND	0.00	0.01	0.00	NS
Pb	Crab	0.02	0.06	0.02	0.01	NS	NS	NS
Pb	Egg	ND	ND	ND		ND	0.00	ND
Pb	Isopod	0.02	0.08	0.03		0.02	0.01	NS
Pb	Snail	0.02	0.04	0.06	0.03	0.02	0.01	0.04
Hg	Mussel	1.25	15.00	ND	0.00	ND	0.00	NS
Hg	Crab	5.63	15.00	0.53	0.51	NS	NS	NS
Hg	Egg	15.22	63.04	3.02		6.50	0.36	4.65
Hg	Isopod	3.75	10.00	0.38		0.62	0.31	NS
Hg	Snail	5.00	7.50	0.60	0.13	0.46	0.30	7.50
Ni	Mussel	0.04	0.06	0.04	0.01	0.04	0.02	NS
Ni	Crab	0.28	0.18	0.23	0.12	NS	NS	NS
Ni	Egg	0.04	0.00	ND		ND	0.00	ND
Ni	Isopod	0.09	0.14	0.16		0.12	0.01	NS
Ni	Snail	0.12	0.10	0.79	1.61	0.13	0.12	0.50
Ag	Mussel	0.41	ND	ND	0.00	ND	0.00	NS
Ag	Crab	1.10	0.61	1.44	3.18			NS
Ag	Egg	ND	ND	ND		ND	0.00	ND
Ag	Isopod	2.06	0.88	46.67		6.90	1.31	NS

Ag	Snail	0.69	ND	ND	0.00	1.85	9.86	183.33
Se	Mussel	1.01	0.81	0.54	0.22	0.41	0.13	NS
Se	Crab	1.88	2.12	1.13	0.47	NS	NS	NS
Se	Egg	0.64	0.91	0.84		0.48	0.03	1.29
Se	Isopod	1.59	1.64	3.25		1.97	0.27	NS
Se	Snail	1.02	0.81	1.69	0.29	0.75	0.27	2.33
Zn	Mussel	0.17	0.16	0.07	0.04	0.07	0.00	
Zn	Crab	0.62	0.54	0.27	0.11	NS	NS	
Zn	Egg	0.41	0.31	0.31		0.15	0.01	0.84
Zn	Isopod	0.44	0.58	1.02		0.30	0.09	
Zn	Snail	0.77	0.51	1.19	0.30	0.40	0.42	1.34

Table A-2. Biomagnification factors (BMF) between prey organisms and eggs. BMFs for sites 3 and 4 are the geometric means of 2-3 subsamples per site. NS= No sample, ND= non-detect.

Element	Type	Site 1	Site 2	Site 3		Site 4		Site 5
		$BAF_{Org-Egg}$	$BAF_{Org-Egg}$	$BAF_{Org-Egg}$	stdev	$BAF_{Org-Egg}$	stdev	$BAF_{Org-Egg}$
As	Mussel	0.06	ND	0.23	0.07	0.15	0.01	NS
As	Crab	0.02	ND	0.04	0.02	NS	NS	NS
As	Isopod	0.02	ND	0.03		0.02	0.00	NS
As	Snail	0.02	ND	0.02	0.00	0.03	0.08	0.02
Cd	Mussel	ND	ND	ND	0.00	ND	0.00	NS
Cd	Crab	ND	ND	ND	0.00	NS	NS	NS
Cd	Isopod	ND	ND	ND		ND	0.00	NS
Cd	Snail	ND	ND	ND	0.00	ND	0.00	ND
Co	Mussel	ND	ND	ND	0.00	ND	0.00	NS
Co	Crab	ND	ND	ND	0.00	NS	NS	NS
Co	Isopod	ND	ND	ND		ND	0.00	NS
Co	Snail	ND	ND	ND	0.00	ND	0.00	ND
Cr	Mussel	2.03	0.87	ND	0.00	All ND	0.00	NS
Cr	Crab	1.11	1.46	ND	0.00	NS	NS	NS
Cr	Isopod	1.18	1.32	0.18		All ND	0.00	NS
Cr	Snail	0.61	2.83	ND	0.00	All ND	0.00	ND
Cu	Mussel	0.21	19.06	0.12	0.03	0.44	0.06	NS
Cu	Crab	0.04	1.25	0.11	0.11	NS	NS	NS
Cu	Isopod	0.03	0.97	0.00		0.01	0.00	NS
Cu	Snail	0.04	1.23	0.01	0.00	0.03	0.14	0.01
Pb	Mussel	ND	ND	ND	0.00	ND	0.00	NS
Pb	Crab	ND	ND	ND	0.00	NS	NS	NS
Pb	Isopod	ND	ND	ND		ND	0.00	NS
Pb	Snail	ND	ND	ND	0.00	ND	0.00	ND
Hg	Mussel	12.17	4.20	ND	0.00	ND	0.00	NS
Hg	Crab	2.71	4.20	6.43	4.83	NS	NS	NS
Hg	Isopod	4.06	6.30	6.43		11.28	0.00	NS
Hg	Snail	3.04	8.41	5.11	1.52	13.42	4.88	1.80
Ni	Mussel	0.96	0.09	ND	0.00	ND	0.00	NS

Ni	Crab	0.13	0.03	ND	0.00	NS	NS	NS
Ni	Isopod	0.39	0.04	ND		ND	0.00	NS
Ni	Snail	0.31	0.05	ND	0.00	ND	0.00	2.67
Ag	Mussel	ND	ND	ND	0.00	ND	0.00	NS
Ag	Crab	ND	ND	ND	0.00	NS	NS	NS
Ag	Isopod	ND	ND	ND		ND	0.00	NS
Ag	Snail	ND	ND	ND	0.00	ND	0.00	ND
Se	Mussel	0.63	1.12	1.65	0.33	1.19	0.15	NS
Se	Crab	0.34	0.43	0.67	0.30	NS	NS	NS
Se	Isopod	0.40	0.56	0.34		0.27	0.01	NS
Se	Snail	0.63	1.12	0.51	0.01	0.65	0.37	0.46
Zn	Mussel	2.45	1.89	5.23	1.53	2.75	0.18	NS
Zn	Crab	0.65	0.57	0.99	0.39	NS	NS	NS
Zn	Isopod	0.93	0.53	0.44		0.53	0.01	NS
Zn	Snail	0.53	0.60	0.27	0.05	0.39	1.37	0.86

Study Data